I. INTRODUCTION

The following study was begun during the summer of 1898 in the laboratory of the Botanical Institute in München upon the suggestion and under the direction of Professor K. Goebel. Only a small beginning was made in München on account of a very brief visit—a few weeks only—to that city. Since then the work has been carried on in New York City.

In undertaking a comparative study of the embryology of the Rubiaceae it is expected to gain a knowledge of the life histories of a number of types sufficient to lead to a clearer understanding of the relationships between the genera of a very highly specialized and polymorphous family. It is intended at the same time to extend the studies into the supposedly allied families, and thus to contribute, if possible, to the solution of the more general problems of relationship among the higher dicotyledons.

In addition to the problems of phylogeny there are those which arise out of the physiological relations existing between the sporophyte and the gametophyte. Modern morphology has acquainted us with the main facts in regard to the process of antithetic alternation of generation in the higher plants, and the general physiological relations which are the outgrowth of interdependence of sporophyte and gametophyte. Out of this interdependence, however, a large number of phenomena of both a structural and physiological character have arisen, offering a field of investigation hitherto neglected, to a great extent, but which latterly has been attracting not a little attention. The phenomena of adaptation in this connection are not a whit less interesting if more difficult to observe. In the present study these adaptive phenomena, so far as the writer has succeeded in observing them, will be set forth. It may, however, very properly be borne in mind that these phenomena are chiefly those of nutrition, and the small

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size of the materials makes any other than morphological evidence difficult to obtain. For example, it is argued from appearances that enzymes or at least zymogens are present in the young endosperm, though that such is actually the case is not proven. Since, however, such substances have been shown to be present and have been extracted from, *e. g.*, the young date seedling * and since the action of the sucking organ of that plant on the hard endosperm is due to the ability to secrete the zymogens of a cellulose-dissolving ferment, we have a working basis for such interpretation.

The position here assumed that morphology must be regarded from a physiological point of view is the one emphasized in 1879, by M. Treub in the introductory part of his "Notes sur l'embryogenie de quelques Orchidées,"† where he expressed the hope that the readers of his paper would agree "que la manière dont les embryons absorbent les substances plastiques mérite certainement d'attirer l' attention, et sourtout d'être élucidées lors de recherches embryogéniques." That this point of view was not new with him, Treub took pains, in a detailed historical treatment, to indicate, though it remained for him to adduce the mass of evidence, embodied in the rich work above referred to, which completely substantiates the value of his position. To this historical review the reader is referred for an account of the growth of our knowledge of the suspensor. The succeeding part of the "Notes" deals with the origin, structure and function of the suspcnsor in eleven genera, including nineteen species of the *Orchidaceae*, in which forms the suspensor is shown to build up for the most part elaborate structures of a haustorial character which serve to absorb nutritive substances to be used by the embryo proper in its development. These structures, which attain the highest degree of specialization in *Herminium monorchis*, *Phalaenopsis grandiflora* and *Stanhopea oculata*, here take the form of tubes ("boyaux") which penetrate into the surrounding tissues of the ovule.

Two years later L. Guignard‡ ('81) published an account of his quite thorough studies on the embryology of the Leguminosae, in

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* F. C. Newcombe. Cellulose Enzymes, Ann. Bot. **13**: 49. 1899.

† Nat. Verh. d. K. Akad. van Wet. **19** 1879.

‡ Recherches d'embryologie végétale comparée: Embryologie des legumineuses. Ann. sc. nat. Bot. VI. **12**: 5–166. 1881.

many species of which the suspensor attains a morphological character quite as striking as that in the *Orchidaceae*. In the *Viciae* especially the suspensor produces a great number of nuclei sometimes not separated by membranes (*Orobus*). Here, however, the endosperm is scanty and the complex suspensor appears to take its place. Guignard suggests that the principal function of the suspensor may be to contribute to the nutrition of the embryo, but admits at the same time that in certain cases the character of that organ indicates that it serves only as a means of attachment for the embryo.

It is of interest in passing to note that Schleiden and Vogel* ('38) saw the complex suspensor in *Lupinus rivularis*, but interpreted it as a development of the pollen tube in accordance with their then maintained view of the origin of the embryo.

The next work with a distinct physiological bearing dealing especially with the function of the suspensor was by S. H. Koorders† who, in 1892, investigated the embryology of the teak with especial reference to the behavior of the embryo in the matter of nutrition. In this plant a most remarkable condition occurs, which, while entirely analogous to that already found by Treub in certain orchids, was shown by Koorders to be morphologically different. The latter investigator found that the suspensor in its proximal, multicellular part is provided at the close of the "Kogelstadium" with "zuigblasen" or haustoria, which, however, are here special endosperm cells which attach themselves to the suspensor and elongate into tubular structures. Basing his inferences on the distribution of food materials in the ovule and embryo, which he found to be cuticularized till the cotyledonary phase was reached, Koorders concluded, also, that the embryo received nourishment through the agency of the suspensor until the appearance of the cotyledons, after which these organs, especially, are concerned in the absorption of foods through their peripheral membranes.

Touching more especially the rôle of the suspensor there appears to be no further work. The adoption of a similar point of

* Beiträge zur Entwickelungsgeschichte der Blutentheile bei Leguminosen. Verhandl. der K. L.-C. D. Akad. d. Nat. **19**: 60. 1839.

† De Kiemotwickkeling van *Tectona grandis* (Djati). Natuurk. Tijdschr. voor Ned. Ind. III, **12**: 139. *pl. 1–8*. 1892.

view by workers on related matters has been productive of some important publications which we may now with profit glance at briefly.

The first paper, important in this connection, was published by M. Westermaier in 1892, entitled "Zur Embryologie der Phanerogamen insbesondere über die sogenannten Antipoden."* He embraced in his observations something like thirty four species of dicotyledons and monocotyledons, and found in the position and character of the antipodal cells and in the distribution of foodstuffs in the ovule, evidence that the view advocated by Vesque† that the antipodal cells are to be regarded as rudimentary, useless, and of only morphological worth is entirely insufficient to explain the facts, and that, on the contrary, we have here to do with a structure which, while in many cases small and apparently insignificant, are really of very considerable importance in the ultimate nutrition of the embryo. Latterly A. Osterwalder‡ ('98) by the study of *Aconitum Napellus* has established and extended Westermaier's view which he himself in a short note§ again expressed as the result of observations on *Alstroemeria* and other plants.

Mottier's ('93) observations on *Senecio aureus* || and Chamberlain's ('95) on *Aster Novae-Angliae* ¶ extended our knowledge of the antipodals by showing that in these two plants as in some other *Compositae* previously studied by others, a greater number of these cells occur than is usual. This multiplication of antipodal cells is of physiological significance, a view which is strengthened by the observations of D. H. Campbell ** ('99) on *Sparganium* and *Lysichiton*, in which these cells are numerous, reaching in the former the number of 150 at least. The author himself suggests that this great development probably indicates an important physiological rôle.

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* Verhandl. der K. L.-C. D. Akad. d. Nat. **56**: 1–39. 1892. (The literature previously published regarding the antipodals is found in this paper.)

† "Nouvelles recherches sur le développement du sac embryonnaire des phanérogames angiospermes." Ann. sc. nat. Bot. VI. 8: 294. 1879.

‡ Beiträge zur Embryologie von *Aconitum Napellus* L. Flora **85**: 254. 1898.

§ Westermaier, Max. "Historische Bemerkungen zur Lehre von der Bedeutung der Antipoden-Zellen." Ber. d. D. B. G. **16**: 214–216. N. 1898.

|| Bot. Gaz. **18**: 245. Jl. 1893.

¶ Bot. Gaz. **20**: 205. My. 1895.

** Bot. Gaz. **27**: 153. Mr. 1899.

The work of perhaps the most interest which has recently appeared is that of Dr. Gabrielle Balicka-Iwanowska † ('99) who has studied the behavior of the embryo-sac in the Scrophulariaceae, Gesneraceae, Pedalinaceae, Plantaginaceae, Campanulaceae, and Dipsaceae. All these forms possess a more or less thickened single integument. Micropylar and chalazal haustoria, which are here for the first time shown to be of endospermic origin, penetrate more or less deeply into the integument which, under such conditions, is said to be devoid of any vascular tissue. Those plants which possess the thickest integument have the most highly developed haustoria, and these in turn have nuclei whose size and appearance show them to be active in nutrition. Their limiting membranes, moreover, are quickly "gelatinized" or become "mucilaginous." The haustoria, in some cases, reach the funicle (*Torenia*) and even the placenta (*Scoparia*). In many species a special nutritive tissue is found in the chalazal region of the ovule and the haustorium is for the most part in direct relation with such tissue. The author does not regard the "tapetes" as merely protective to the embryo-sac, as Hegelmaier held, but holds that they probably secrete a ferment and exercise a digestive function. The antipodal cells, when present, are regarded as having only a less important mission, and are at best evanescent.

While Dr. Balicka-Iwanowska's paper was in preparation at München, Mlle. M. Goldflus* ('99), was engaged in the laboratory of the University of Geneva, in a similar investigation with especial reference to the *Compositae*. Her conclusion as to the physiological value of the "epithelium," the "tapetes" of Balicka-Iwanowska, agrees with that of the latter, an opinion favored, though not fully subscribed to, by Schwere. She furthermore attributes to the antipodal cells considerable importance as agents in nutrition. These cells attain in some of the species studied a considerable size (*Leucanthemum lacustre*, *Chrysanthemum Leucanthemum*, *Helianthus Maximiliani*) and are interpreted as a haustorium (suçoir) inasmuch as they penetrate the axial part of the ovule, and stand in relation with a mass of conductive tissue which

† Contribution à l'étude du sac embryonnaire chez certain Gamopetales. Flora, 86: 47. 1899.

* Sur la structure et les fonctions de l'assise épithéliale et des antipodes chez les Composées. Jour. de Bot. 12: 374. 1898; 13: 9, 47, 87. 1899.

in turn is in a similar relation with the vascular bundle. Mlle. Goldflus bases her opinion not alone on the position of the antipodals, for she finds in the cyanophily of these cells evidence that they represent "l'intermediaire entre le sac embryonnaire et les substances digestibles élaborées par l'ovule."

The *Rubiaceae* in particular have received very little attention. Schleiden * appears to have been the earliest observer whose observations may be regarded as pertinent to the present work. He described the ovule in the *Rubiaceae* as anatropous (gemmula anatropa) and as consisting of a naked nucellus (nucleus nudus), *i. e.*, without any integument (p. 304, *l. c.*). As Warming † points out, it is easily understood how the single thick integument with the very delicate micropylar canal would have misled the earlier observers. These results seem to have remained without modification till the appearance of his "Grundriss," ‡ in which, however, it is further stated (p. 163) that in many *Rubiaceae* the integument remains at least as a thin skin which clings to the endosperm, from which it easily falls off in tatters, as in *Coffea*.

The work of Hofmeister, § as is well known, covered a very large field, and of necessity the treatment of any given group was relatively brief. The results of his work on the *Rubiaceae* are quite brief and faulty. It would, however, be very unfair to criticise him, on account of the difficulties presented by the materials, especially in view of the methods then used, methods which, by the way, in the hands of a master like Hofmeister gave surprising results. According to this pioneer in embryology the antipodals are absent from *Galium*, *Asperula* and *Crucianella*, in which the embryo-sac is attenuated and swollen at the micropyle end. The embryo-sac is quickly filled with endosperm after fertilization has taken place. Within the endosperm, the proembryo develops by forming transverse walls. The cells of a filamentous structure thus formed produce numerous branches made up of short series of cells of which the outer-

* Einige Blicke auf die Entwickelungsgeschichte des vegetabilischen Organismus bei den Phanerogamen. Wiegmann's Archiv. 3: 289, 414. 1837.

† De l'ovule. Ann. sc. nat. Bot. VI. 5: 177. 1878.

‡ Grundriss der Botanik. Leipzig. W. Engelmann, 1850.

§ Neure Beobachtungen über Embryobildung der Phanerogamen. Pringsh. Jahrb. 1: 82. 1858.

most is rounded and swollen. The cells from the middle of these branches send out secondary branches here and there, so that the proembryo as a result of this richness of branching simulates a bunch of grapes. Out of the lower end of this mass projects the primary cell row of the proembryo whose terminal cell develops into the embryo proper. Thus Hofmeister's description of the suspensor in the genera above named, one in the main correct, but requiring modification, as will hereafter be seen. He passes over without criticism and accepts as final the opinion which he attributes to Schleiden that the endosperm breaks through the integument.

In *Houstonia* and *Spermacoce* the embryo-sac is of less slender form and somewhat cylindrical, and here, on the other hand, the antipodal cells are present. The embryo-sac is here also soon filled with endosperm, although the proembryo remains a very simple thread of cells from the last of which the embryo proper arises.

It is unfortunate that no figures of these forms are given so that it is difficult to estimate exactly the value of the author's descriptions.

The only other plant of the *Rubiaceae* which has received any attention is the coffee (*Coffea arabica*), but such work as has been done does not touch upon the ground of the present paper except in a remote way. An account of the literature of this subject may be found in the footnotes of an article by T. F. Hanausek.*

I desire at this point to express my appreciation of the kindness of Professor Ignatius Urban in putting the collection of growing *Rubiaceae* in the Botanical Garden at Berlin at my disposal, and my gratitude to Professor K. Goebel for his generosity in extending to me the courtesy of the laboratory and gardens of the Botanical Institute in München during six weeks of the summer of 1898, and in expending much of his own time in discussion and suggestion when he could ill afford, on account of pressing duties, to do so.

* Ueber symmetrische und polyembryonische Samen von Coffea arabica L. Ber. D. B. G. **13**: 73. 1895.

II. DESCRIPTIVE

Vaillantia hispida

Vaillantia is a small genus of the *Galieae* containing only two annual species, indigenous to the Mediterranean region. The plants are monoecious; the flowers are borne in threes supported by a single broadened spiny stalk. Of the three the middle is pistillate, the two lateral staminate. The three-flowered peduncles are in four vertical rows alternate with the four rows of leaves. The regularity and perfect radial symmetry of the species studied render it easy to section a growing tip so as to cut all the ovules of two opposite rows longitudinally.

The material was obtained at the Botanical Garden at Berlin.

THE ORIGIN AND DEVELOPMENT OF THE NUCELLUS

At the period of the development of the pistillate flower when the four corolla lobes have met above the hollowed out receptacles and overarch the stamens, which are as yet merely rounded knobs at the angles of the sinuses of the corolla, four elevations lying in the same vertical plane make their appearance near the base of the ovary. The outer two of these are ridge like, and by their mode of growth meet later in the transverse plane, fuse, extend upwards to form the two styles, and downward to form a partition which divides the originally unilocular ovary into two chambers. The two others are papilliform and lie on either side of the center of the floor of the ovary. These are the nucelli. As they develop, the partition above referred to passes down between them and separates them. The lower edge of the partition finally fuses with the tissues between the funicles of the two ovules, making the ovary bilocular.

We may now turn our attention to the nucellus. For a short time its growth is directly upwards. (Pl. 1. Fig. 1.) There is a well marked epidermis, and the cells are quite filled with dense cytoplasm and large nuclei. More rapid cell division upon the inner side, however, soon causes the apex of the nucellus to be directed toward the floor of the ovary. When the bending of the ovule is complete, and its definitive condition reached, the micropylar

end of the ovule lies in a lower horizontal plane than does the base of the funicle. The micropyle and embryo-sac lie in a curved line, and the funicle remains short. The ovule is therefore not of the strictly anatropous type, but is campylotropous, approaching the anatropous condition. (Pl. 3. Fig. 1.) During the process of inversion the longitudinal axis comes, of course, to lie in the horizontal plane, and about this time a number of the hypodermal cells under the apex of the nucellus elongate in a direction parallel with the longitudinal axis of the nucellus. (Pl. 1. Fig. 2.) These cells, about 12 in number, constitute the archesporial tissue, and are recognizable at first only by their size. Their subsequent changes will be described below. The large number of sporogenous cells here found to constitute the archesporium recalls the condition in *Rubus caesius*, *Geum strictum* and *Sanguisorba officinalis*, in which, according to A. Fischer* ('80) numerous sporogenous cells arise. A similarly large number of these cells has also been found in *Casuarina*,† in *Loranthus*‡ and in *Ranunculus*.§ This character, which appears in several widely separated families is probably therefore not to be regarded as a persistent primitive character in the Rubiaceae at any rate, but one having a physiological meaning, as will be shown later. The sporogenous cells now elongate, and their elongation is accompanied by periclinal division in the epidermis and subadjacent cells of the tissue about the apical part of the nucellus. (Pl. 1. Fig. 3.) Only in the columnar epidermal cells immediately beneath which lie the sporogenous cells there occurs no division, periclinal or otherwise. These, therefore, remain as a cap of cells, about which arises the integument at first as a low ridge, but gradually growing over the nucellar cap and forward so as to form a canal which I shall call the micropylar canal, the outer end of which forms, in the definitive ovule, the micropyle.

The manner in which the integument has its origin recalls vividly the figures by Warming || ('78) of the nucellus of *Thesium*, one of the *Santalaceae*. In the body of the paper the author asks the question "Are these ovules in which the nucellus is not cov-

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* Jenaisch. Zeit. f. Nat. **14**: 122. 1880.

† Treub, M., Ann. de Buit. **10**: 145. 1891.

‡ Treub, M., Ann. sci. nat. Bot. VI. **13**: 274. 1882.

§ Coulter, Bot. Gaz. **25**: 73. 1898.

|| De l'ovule. Ann. sc. Nat. Bot. VI. **5**: 177. 1878.

ered by an integument," and recounts the forms in which, according to earlier students, the nucellus is naked. Some reference to this point has already been made in the introduction. In endeavoring to contribute to the answer of the question, Warming studied the development of the nucellus in the type above mentioned, and found that the epidermal cells at the apex of the nucellus, and therefore, those which cap the elongated cells which lie in the axis of the structure, have contents more granular than the remaining epidermal cells, and also that the epidermal cells surrounding these capping cells divide tangentially and "il se développe ainsi une assez forte couche de cellules de provenance épidermique qu'on doit considérer comme un tégument." His description with the exception of the reference to the more granular contents of the middle epidermal cells is exact enough to apply to the *Galieae*, and one cannot resist the conclusion that his interpretation of this collar of tissue as a rudimentary integument is correct. Guignard, * seven years later, published a fuller account of the behavior of the nucellus and embryo-sac of *Thesium* in which, however, he entirely overlooked Warming's work and suggestion. Guignard here makes no reference to the tangential divisions seen by Warming, nor does he show any such in his figures. Indeed he takes the position (p. 189, l. c.) that there is no integument at all in *Thesium*, though it must be said that in making the statement he is referring to the seed, rather than to the young ovule.

The bottom of the micropylar canal is formed of the few epidermal cells capping the archesporium (Pl. 1, Figs. 5, 6, 8). The walls also are formed by an epidermis continuous with and having its origin in the outer epidermis of the ovule. The endodermis †

* Observations sur les Santalacées. Ann. sc. nat. Bot. VII. 10: 181. 1885.

† This layer has its homologue in the Compositae in the inner cell-layer of the integument which, according to Hegelmaier (Ueber die Keimsack einiger Compositen und dessen Umhüllung. Bot. Zeit. 47: 805, 821. 1889), comes to surround immediately the embryo-sac after its absorption of the nucellus is complete. For this layer Hegelmaier proposed the term *endodermis*, the types under observation and figured being *Helianthus annuus*, *Bidens leucantha* and *Zinnia tenuiflora*. Schwere (Zur Entwickelungsgeschichte der Frucht von *Taraxacum officinalis* Web. Ein Beitrag zur Embryologie der Compositen. Flora 82: 32. 1896), however, working on *Taraxacum officinalis*, regards the layer surrounding the embryo-sac as having its origin in the outer cell-layer of the nucellus, "bei *Taraxacum* gehört diese Schicht thatsächlich zum Nucellus," a conclusion which is out of harmony with the prevailing view. For this

thus formed does not, however, have the specialized character which has been found to be so common in the *Gamopetalae*. It is not cutinized, and, as will be seen, breaks down very readily under the action of the developing embryo-sac.

Meanwhile the archesporial cells are undergoing considerable changes which will now be described.

At the time of rapid elongation of the nucellus, the archesporial cells, too, increase in length. This is accompanied by a good deal of change in the appearance of their cytoplasm and nuclei. The cytoplasm which at first and for some time is granular, becomes more and more fibrous in appearance. The fibers run approximately lengthwise. The granular character is still visible, but the stringiness becomes more marked as the cells themselves elongate (Pl. 1, Figs. 3–8). When they reach their maximum length they are spindle-shaped and appear to run under and above each other in a most perplexing fashion. Their separating membranes become less and less distinct, until they cannot be made out, and I believe them to be almost, if not entirely, absorbed. The nuclei, too, rapidly increase in size, while the chromatin, granular at first, runs through various changes, unnecessary to describe here, which are preparatory to mitosis.

It should be pointed out here that the nucellar tissue next to the archesporial tissue on its inner side is made up of cells which lengthen much more than those of the rest of the nucellus (Pl. 1, Fig. 5). The innermost of these are, indeed, as a usual thing, as long as the archesporial cells and appear to partake to a great degree of their characters. That the nucellar cells next to the archesporial cells may approach the latter in character has been noted by Coulter (l.c.) in *Ranunculus*. They may without doubt be regarded as underdeveloped sporogenous cells, which in this case disintegrate, while their substance probably contributes to the growth of the archesporium.

When the archesporial cells as above mentioned have reached

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layer he proposed the term *endothelium*, a term which is evidently inappropriate. Schwere's figures as to this detail are very unsatisfactory and unconvincing, while it is quite evident from Hegelmaier's drawings that the layer to which he gave the name *endodermis* is in reality what he thought it to be. This layer is, moreover, an *inner* layer, so that *epithelium*, the term adopted by Mlle. Goldflus, seems to me a less appropriate term, because more general.

their greatest elongation, mitosis takes place. I shall take the opportunity of describing the cytological details in a separate paper. For the present purpose it is enough to say that so far as my own observation goes four megaspores result from the division of each primitive cell. The appearance of the mass of cells which results from the division of the archesporial cells is very similar to that presented by similar cells arising in the same manner in *Helianthemum Rhodax* as described and figured by A. Fischer* according to whom two or three "mother cells" are in this form cut off, which divide each into four or sometimes six daughter cells (megaspores) one only of all these resultant cells becoming the mother cell of the embryo-sac, the rest suffering disorganization.

Several or all of the archesporial cells may divide; thus may many megaspores arise,among which there is a considerable discrepency in size (Pl. 1, Fig. 9). Of one quartette arising from one mother cell, the lowest cell may be the largest; in another, one of the middle ones; then again the uppermost may be the largest. I have not been able to determine whether there is any constancy in the selection of a megaspore to be the mother cell of the embryo-sac. The appearance of such material as I have examined seems to favor the opinion that any one of them, but more especially one of the end cells of the quartette, may be chosen.

As a rule only *one* of all the megaspores actually germinates. Exceptionally in other genera to be hereafter described, I have found two embryo-sacs completely formed and lying parallel; one of these usually lies farther forward than the other, and this one is functional.

THE DEVELOPMENT OF THE EMBRYO-SAC

At the completion of megaspore formation, the chosen megaspore commences a migration along the micropylar canal formed as above described by the forward growth of the integument. This migratory movement of the functional megaspore appears to be unique, for I have not been able thus far to find any reference in the literature which shows that such migration has hitherto been observed. The cap of epidermal cells which overlies the archesporium

* Zur Kenntniss der Embryosackentwickelung einiger Angiospermen. Jenaische Zeitschr. 14: 90. 1880.

seems to be a barrier to any such migration, for the migrating megaspore, so far as I have been able to determine, pushes its way to one side, as shown in Pl. 2, Fig. 1. It is reasonable to conclude that these capping cells are in a short time destroyed, for in later stages they are not usually seen. I have seen these cells once in one species of *Galium*, when the embryo-sac was mature.

The nucleus of the migrating megaspore, after passing this barrier, comes to lie in the micropylar canal about midway its length, while the accompanying cytoplasm lies in a thin layer on the walls of the canal, and so forms a large vacuole at each end of the nucleus. The whole megaspore is, roughly speaking, hour-glass-shaped (Pl. 2, Fig. 1), and its nucleus lies at the constriction. The enlarging megaspore encroaches laterally on the integument, which now for the first time is broken down. The endodermis gives way first, and is recognized a little later only by collapsed walls and the nuclei, which are the last elements of the cells to disintegrate. The persistence of nuclear substance under the action of digestive ferments suggests that the tissue which behaves thus is undergoing digestion. This is the view taken by Mlle. Goldflus (*l. c.*) in describing the behavior of the integumental tissue about the base of the embryo-sac in the *Compositae* the internal part of which is dissolved by "ferments secrétés probablement par les cellules epithéliales."

At the constriction in the hour-glass-shaped megaspore the first division of the megaspore nucleus takes place, after which one of the daughter nuclei passes forward to the micropylar end of the cavity. When it reaches this point the second division takes place in both nuclei (Pl. 2, Fig. 2). There are now four. The egg apparatus arises in the usual fashion at the micropylar end (Pl. 2, Fig. 3, 4). In the antipodal region a more unusual state of affairs occurs.

THE ANTIPODAL APPARATUS

The division of the antipodal nuclei, now two in number, results in four, one of which is, in the usual manner, concerned in the formation of the endosperm nucleus. This passes forward to coalesce with the corresponding nucleus from the egg region. Of the three which remain, one enlarges considerably and migrates

backwards towards the chalazal region. Its proper cytoplasm becomes cut off by a transverse wall from the upper part of the embryo-sac (Pl. 2, Fig. 4). The other two antipodal cells surround themselves by walls, and take ultimately a lateral position. They are small and unequal in size and are usually more or less collapsed and apparently of little or no further value; and they overstain in the manner of disintegrating cells. Not so, however, with the odd, basal antipodal, for in these forms (*i. e.*, *Vaillantia*, *Galium*, etc.), it appears to have a distinct and important physiological rôle.

It has been supposed, since the publication of Hofmeister's classical researches on the embryology of the Phanerogams (*l. c.*), that antipodals were absent in those forms of the *Rubiaceae* studied by him, excepting *Houstonia* and *Spermacoce*. I have confirmed Hofmeister's view as to *Houstonia*, but in addition I am prepared to show that antipodal cells are present in all *Rubiaceae* up to this time studied by me. The smallness of the structures in these plants, and especially the attenuated character of the basal antipodal, together with the similarity of the other two to the adjoining disintegrating integumental cells will readily account for the oversight.

The basal antipodal cell is composed at first of cytoplasm which is tenuous, scanty and difficult to see. The nucleus comes to rest on one side of the cavity some distance removed from the transverse wall.

Gradually the cytoplasm becomes more pronounced, stains more deeply and fills the elongating chalazal end. The apex becomes somewhat expanded into a knob which, as the cell lengthens, is plunged into the mass of megaspores. These are still evident, but are now on the way to disintegration as is attested by the fact that they lose all structure and become black and opaque with haematoxylin, often making it very difficult to recognize the apical, knobbed part of the long antipodal. At the time when the basal antipodal is first cut off, there is a large mass of proteinaceous food in the place of the once archesporium, which as just stated, stains very deeply. As the embryo-sac as a whole reaches maturity, before the flower opens, the cytoplasm about the endosperm nucleus becomes abundant and dense, and the archesporial food-

mass is coördinately reduced, while the basal antipodal shows its maximum development during this period. The probe-like end, which, I believe, serves as a haustorium, at this time and somewhat later is filled with cytoplasm which appears finely reticulate, while the broad end which is adjacent to the upper part of the embryo-sac is occupied by a large vacuole. This large antipodal must, therefore, stand in an important relation between the food supply derived from the archesporium and the endosperm cell, and is probably active in the transportation of the food from the former to the latter. The megaspore mass thus forms a sort of nutritive tissue, partly analogous to that described by Dr. Balicka-Iwanowska (*l. c.*) as occurring in the region of the integument near the lower ends of the embryo-sac in the forms studied by her.

The archesporial tissue is now seen to bear a nutritive relation to the embryo-sac, which by this time has reached maturity.

THE DEFINITIVE EMBRYO-SAC

We may now pause to sum up the characters of the mature embryo-sac.

In shape it is fusiform and much attenuated at the lower end. The upper part, comprising one third or more of the entire length, contains the egg apparatus and the endosperm nucleus, and constitutes the expanded part of the embryo-sac. The fusion of the polar nuclei takes place at about the middle of this part of the embryo-sac, but at the time of fertilization the resultant nucleus comes to lie immediately against and partly surrounding the egg nucleus. The migration of the endosperm nucleus has been noticed by Mme. Balicka-Iwanowska (l. c.) in *Digitalis* and other related forms in which the embryo-sac is more or less attenuate. At the time it reaches the egg, the nucleolus has nearly three times its original diameter. Its size relative to that of the egg nucleus is shown in Pl. 2, Fig. 8a. The nucleus itself is larger than the egg nucleus, but the discrepancy in size is more striking between the nucleoli. This movement and growth of the endosperm nucleus must be interpreted physiologically; it is in all probability in some way connected with the nutrition of the egg.

The antipodals are three in number; two of these are small

and relatively inconspicuous; the third and basal, is very long, being on the average about two thirds the length of the whole embryo-sac, though it may, in exceptional cases which I have noted, attain a considerably greater length. At its expanded upper end it is separated from the upper region of the embryo-sac by a very thin transverse wall. Its lower end is somewhat knobbed, and is embedded in a mass of degenerating megaspores. The position of the nucleus of this antipodal is always lateral and about one fourth to one half the cell's length from the upper end (Pl. 2, Figs. 7–11). It is large and prominent, and evidently active. Its function has already been discussed. The fate of the antipodals will be discussed somewhat later.

DEVELOPMENT OF THE EMBRYO

After the egg is fertilized, its vacuole enlarges considerably, pushing the cytoplasmic end rather more deeply into the endosperm stuff. Immediately the endosperm nucleus commences to divide so that before the first division of the fertilized egg takes place 20 to 30 endosperm nuclei are present (Pl. 2, Fig. 10). The proembryo now grows rapidly by the successive occurrence of approximately transverse divisions. When about five cells have been formed, the proembryo becomes bent to one side and the other. This is due to the lateral enlargement of certain of the suspensor cells, as a result of which they bulge out into the endosperm which is now of some bulk. In these enlarging suspensor cells, the cytoplasm becomes vacuolated to a considerable degree. These facts and later developments indicate that these cells are concerned in the absorption of foods in solution from the endosperm, which, however, is not broken down by the enlarging suspensor cells. The cells of the end of the embryo away from the micropyle are always smaller during the earlier development of the proembryo, while they get successively larger toward the micropylar end where the basal cell tapers off acutely, giving the young proembryo something of a spindle shape (Pl. 4, Figs. 1, 2).

When the proembryo attains the number of about six cells, longitudinal divisions occur in some of the suspensor cells. The divisions sometimes occur in two planes, and the resulting elements continue to grow and bulge out further into the endosperm and

form haustorial appendages. In some cases when two longitudinal divisions have taken place in a single suspensor cell, a pair of the resulting quadrants grow out together, forming double haustoria (Pl. 4, Fig. 5). Again the haustorial cells may divide transversely ; this occurs only in the region near the embryo proper.

These haustoria, for such I regard them, are at first pronouncedly vacuolated, but as the embryo ages the vacuolation disappears, and they become densely filled with cytoplasm. After the embryo proper begins its development, the basal suspensor cell elongates considerably, increasing its length by a half and becomes less vacuolated. Its micropylar end becomes crushed by the growing endosperm, which now begins to grow into the micropylar part of the thickening integument, leaving the embryo in a more central position (Pl. 4, Figs. 9, 10). Later, the basal cell and even one or two of its neighboring suspensor cells become disorganized, but the time at which this occurs is not constant, for while I was not able to find these cells in the preparations from which Figs. 5 and 6, Pl. 4, were drawn, I did find them in another specimen of such an age as the one shown in Pl. 4, Fig. 7.

After the embryo proper has differentiated itself from the suspensor, the latter may be seen to be divided clearly into two regions which differ very markedly in structure. These two regions may be termed the micropylar and embryonal. The latter is made up of half a dozen disc-shaped cells which are very much flattened longitudinally, and show no outgrowths and are not vacuolated, and they remain so during the entire embryonic history. The cells of the micropylar region, on the other hand, become larger and thus increase their absorbing surface. When they reach their maximum development the suspensor when dissected out resembles a "bunch of grapes." These outgrowths, to which we have ascribed a haustorial function, are not unlike the suspensorial haustoria (Keimträgerblasen, boyaux) described by Treub in the *Orchidaceae* and by Hofmeister* ('49) and Guignard (*l. c.*) in *Sutherlandia*, and are analogous to the "zuigblasen" occurring in the teak (*Tectona grandis*) described by Koorders ('92). The upper part of the micropylar region, as shown above, becomes less important and is finally lost by absorption, so that the embryo

* Die Entstehung des Embryo der Phanerogamen. Leipzig, 1849.

with its suspensorial haustoria, to which it is attached by the short row of disc-shaped cells, comes to lie entirely within the now large mass of endosperm (Pl. 4, Figs. 9, 10). The structures above described are probably to be regarded as an adaptation for the ready absorption of food for the rapid growth of the embryo. The shortness of the cells of the embryonal region of the suspensor favors the passage of food to the embryo. Whether or not the embryo is capable of absorbing food through its own surface is a question which I am unable to answer at this time. Koorders ('92), working on *Tectona*, found that the embryo till the completion of the "Kogelstadium" possessed cuticularized walls, thus preventing the absorption of food. If this be true of *Vaillantia* and its close relatives, we have a complete explanation of the significance of the haustoria. But even if the embryo is not cuticularized, it is not improbable that the amount of absorbing surface on the embryo proper alone is insufficient for its rapid development, and it may be that in this lies the explanation of many features in other forms not yet cleared up. For example, I have found that the basal cell in *Capsella*, figured years ago by Hanstein, and copied in almost every text-book, actually pushes its way into the adjacent tissue of the inner integument in the same manner that the embryo-sac itself, by its growth, in many cases encroaches upon the surrounding tissue. This fact indicates that the suspensor, in forms in which it reaches so pronounced a development, as in the *Cruciferae*, *Tropaeolum* and others, is of importance in absorbing nourishment, an opinion which we must credit to Meyen ('39).* I believe this action of the suspensor in the surrounding tissue to be correlated with the rapid growth of the embryo in *Capsella*. Before drawing any conclusions of this kind, however, it will be necessary to obtain many more data.

There can be little doubt that the rapid development of seeds is an important adaptive feature, and the study of structures which are correlated with this ability will be a fertile field for further study. Such haustorial structures as those found in the *Orchidaceae* may be correlated with the meagre integument which means a meagre store of food immediately at hand.

The further development of the embryo, which brings the

* Neues system der Pflanzen-Physiologie 3: 331. 1839.

cotyledons into view, is accompanied by the dissolution of a zone of endosperm surrounding the embryo. The cells become mucilaginous, and the embryo is bathed in nutrient substance. The relative increase of absorbing surface by the development of the cotyledons is accompanied by the gradual loss of the haustoria of the suspensor, of which there may be found, at the time of the ripening of the fruit, only a few of the disc-shaped cells. The last of these plays a small part in the formation of the radicle.

THE INTEGUMENT

The earlier development of the integument has already been described. Some time before complete maturity of the embryo-sac is reached the cells which form a zone (*d*, Fig. 1, Pl. 3) about the attachment of the funicle to the ovule become large and rounded (Pl. 3, Fig. 1, *d*) and their contents become strongly vacuolated. These cells contain a good deal of starch at quite an early condition of the ovule, and when the accumulation of starch in the integument commences, the process has its beginning here. At the end of the vascular bundle when it reaches this zone of enlarged cells, is found a mass of cells which gives the bundle a club-shaped appearance in longitudinal section. These cells, like those of the leptome of the bundle, are rich in contents, and form a center for the distribution of food, which is received by the large-celled zone above referred to, the function of which appears to be the preparation of food and the regulation and distribution of the supply to the meristematic zone. The latter is made up of smaller cells (Figs. 1*b*, 1*c*, Pl. 3), in which, after fertilization multiplication begins. Their dividing walls are periclinal, and there results a rapid thickening of the integument, with a corresponding increase of the ovary wall, accompanied by secondary changes leading to the formation of a schizocarp. Within the meristematic zone the integumental tissue shows signs of disintegration, and this process appears to be as rapid and complete in the tissue surrounding the basal antipodal as in that about the endosperm region. We may notice in this connection that the mass of disintegrated megaspores has after fertilization and before the first division of the proembryo almost disappeared (Pl. 2, Fig. 9; Pl. 3, Fig. 3) and the antipodal cells are difficult to

see. In some genera they persist for some time, and vestiges may be seen after a large mass of endosperm has been formed. After the megaspore mass is absorbed, the basal antipodal appears to have no further function.

When the embryo starts to develop a small amount of starch is found in the integument, principally in those cells above referred to which form a zone between the funicle and the meristem of the integument. A very few granules are also to be found in the cells immediately about the embryo-sac. As the seed approaches maturity the starch accumulates first in the peripheral cells until all those cells of the integument which remain are replete.

THE GROWTH OF THE ENDOSPERM

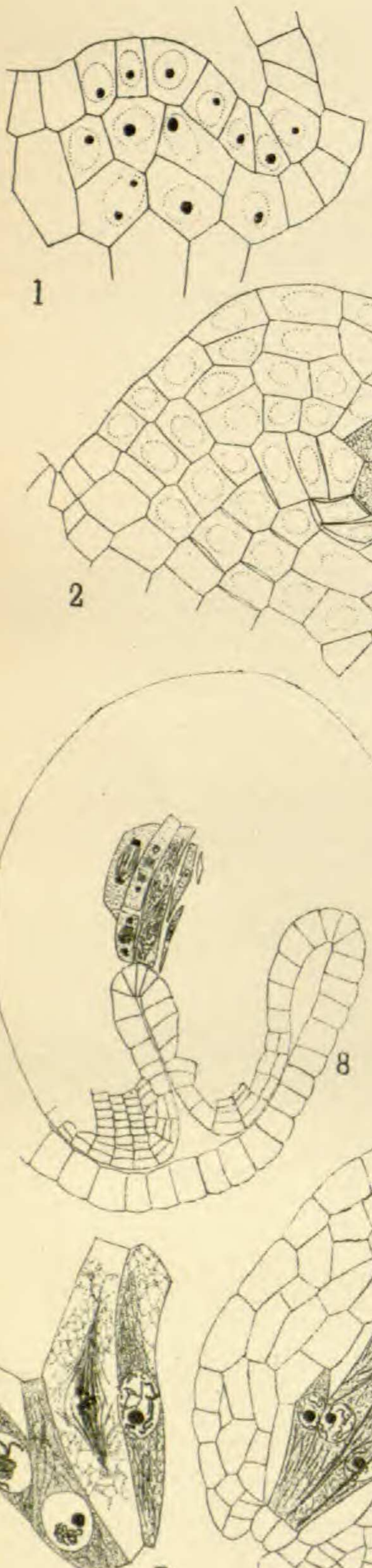
During the first division of the endosperm nucleus the endosperm enlarges, becoming rounded, and encroaches upon the adjacent tissue of the integument, the cells of which become flattened and give way. At first, separating walls between the cells are not formed. The nuclei are not parietal, nor do they ever become so. The endosperm mass is always solid. A little later the parietal cells become much more densely filled with cytoplasm and stain more deeply (Pl. 3, Figs. 2*b*, 2*c*). This is especially true of those cells on the side of the endosperm mass facing the longitudinal axis of the ovary, which are the earliest to become dense. It is on this side that the thickness of the integument is the greatest and where the food is most abundant. After the endosperm approaches its limit of growth, these are the last of the peripheral cells to lose their dense character. The inner cells of the endosperm are strongly vacuolated, the vacuolation becoming more pronounced towards the center, where the cells ultimately break down and leave a cavity within which lies the now rapidly maturing embryo. The peripheral cells are digestive, I believe, and absorptive, for the integuments break down in advance of the growth of the endosperm, and the cell walls of the disorganized cells become thickened. These thickened walls stain readily and deeply with safranin, while the walls of the normal cells show much less affinity for that stain.

As the endosperm ages it becomes concave on its inner face, and dipping into the concavity is a mass of integument which

remains undigested at the maturing of the fruit. (In Pl. 3, Fig. 2.) This mass is continuous with a thin layer of integument two cells in thickness, which surrounds the endosperm. The latter does not, contrary to the opinion of Hofmeister, break through the integument, so that the envelopes of the mature fruit consist of the wall of the ovary lined by an integument (Pl. 3, Figs. 2, 2*a*) two cells thick and loaded with starch. The endosperm cells up to a time when the embryo is large and approaching maturity, have thin walls. Starch accumulates more and more till the cells are gorged. Just before the complete maturity, however, the walls thicken so that in the mature seeds the principal foods stored in the endosperm are reserve cellulose* and starch.

The central part of the endosperm is, up to the completion of the sphere stage of the embryo, continuous, so that the embryo lies in direct contact with the endosperm cells. When, however, the cotyledons begin to appear, the endosperm immediately surrounding the embryo breaks down, so that the embryo comes to lie freely in a cavity which is filled with fluid materials. The behavior of the endosperm in this regard, as also the integument during the advance of the endosperm upon it, indicates the presence of digestive agent secreted by the embryo on one hand and by the endosperm on the other.

*The term 'reserve cellulose' is used here provisionally. The exact nature of the substance in the *Galieae* is, I believe, not yet determined.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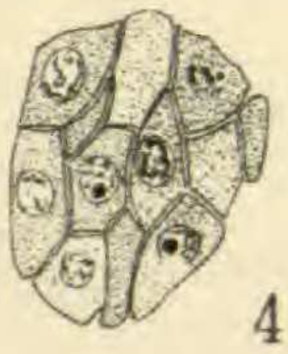
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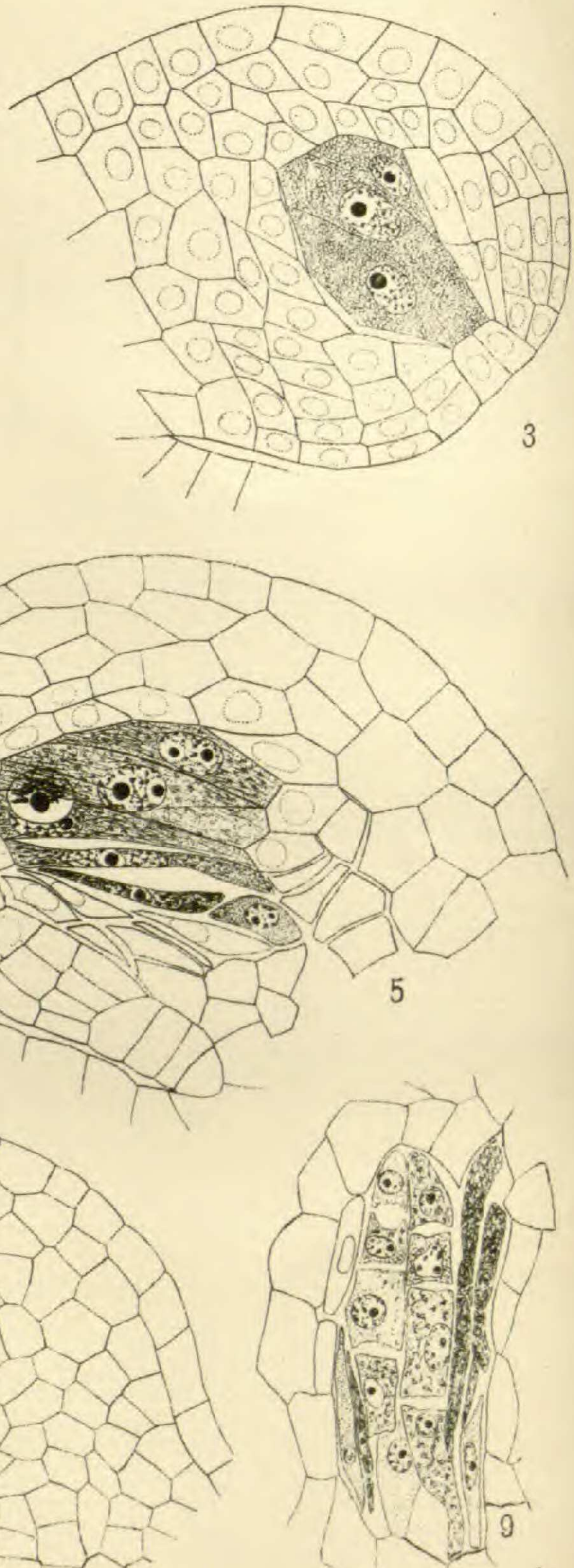
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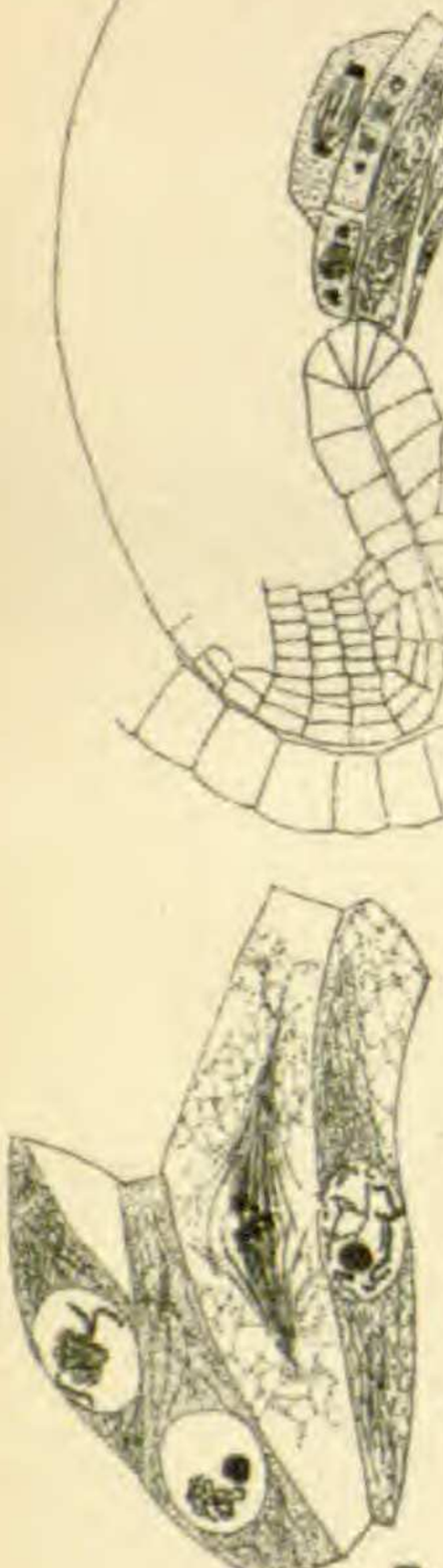
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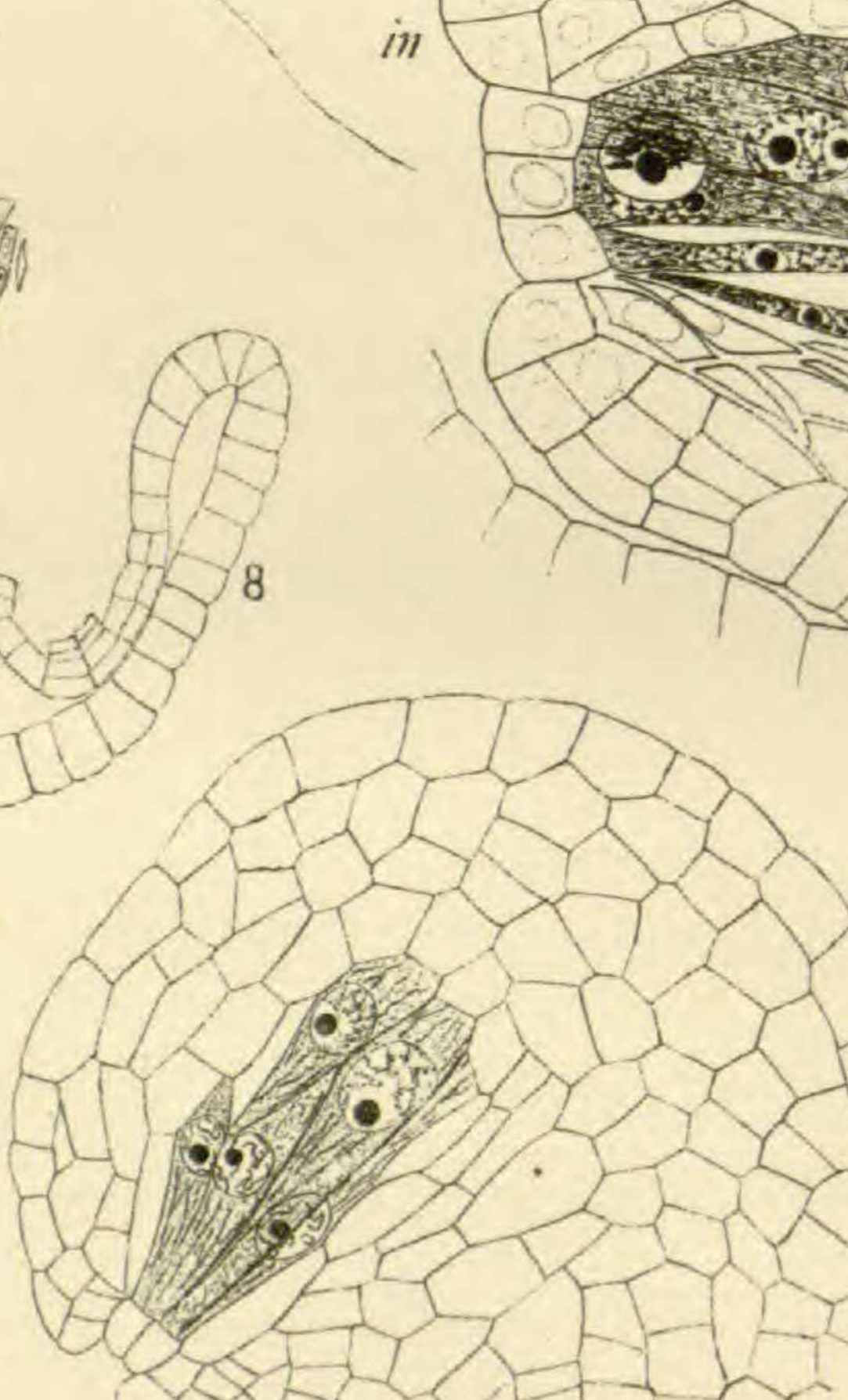
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VAILLANTIA HISPIDA

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Explanation of Plate I

Fig. 1. The fundament of the nucellus, × 670.

Fig. 2. The archesporial cells, of which only two are seen in the section, have begun to be differentiated. They apparently underlie only one epidermal capping cell.

Fig. 3. The archesporium more strongly developed. The periclinal divisions about the capping epidermal cells mark the beginning of the growth of the integument.

Fig. 4. Transverse section through archesporium; it is composed in this instance of twelve cells, × 670.

Fig. 5. Integument is well marked; maximal elongation of archesporial cells; disintegration of some of the inner cells has begun, × 670.

Fig. 6. Somewhat older condition of the ovule in which the archesporial cells are preparing for division; micropylar canal is begun, × 472.

Fig. 7. First division of the archesporial cells. The arrow is directed toward the position of the micropyle, × 670.

Fig. 8. A still more advanced condition of the ovule in which the micropylar canal is deep. The second division is taking place in some of the archesporial cells, × 315.

Fig. 9. Definitive megaspore. On the right one sees imperfectly formed and degenerating megaspores, × 670.

Explanation of Plate 2

Fig. 1. The embryo-sac mother cell has migrated down the micropylar canal, having passed to one side of the nucellar cells which cap the archesporium. The remaining megaspores are beginning to disintegrate, × 670.

Fig. 2. Embryo-sac with four nuclei, × 670.

Fig. 3. Embryo-sac with eight nuclei. One of the antipodals is cut off from the remaining seven nuclei by a transverse wall, × 670.

Fig. 4. Egg apparatus and antipodals definite. Approach of polar nuclei, × 472.

Fig. 5. Fusion of polar nuclei completed. The position of endosperm nucleus is near the middle of the sac, × 472.

Figs. 6 and 7. Approach of endosperm nucleus toward the egg, and their concomitant increase in size, × 472.

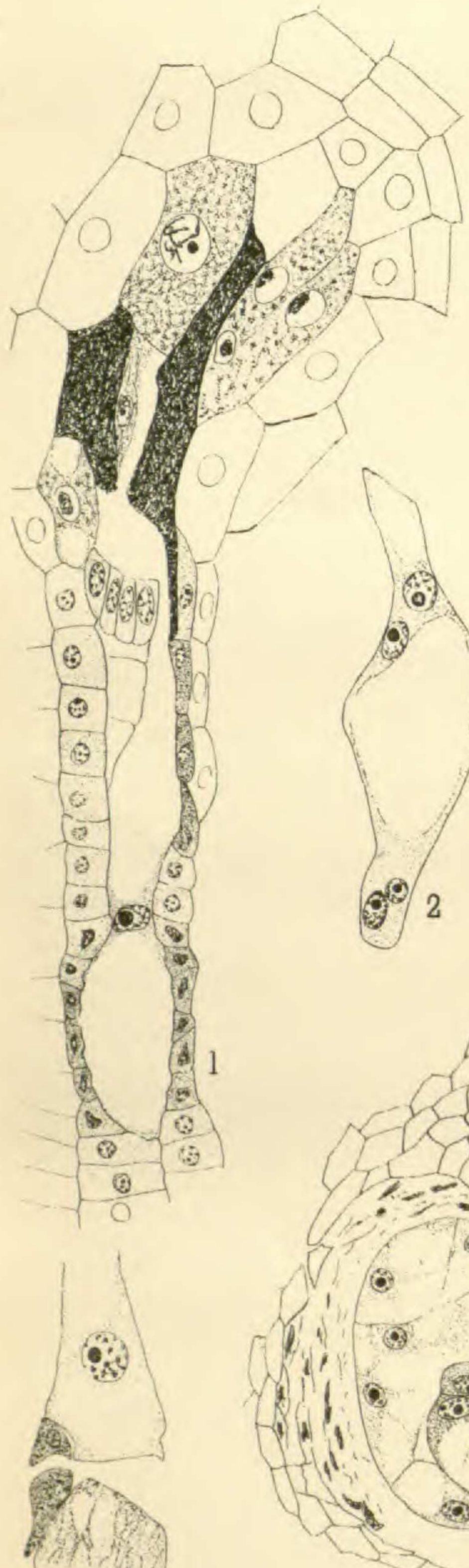
Fig. 8. Mature embryo-sac. The endosperm nucleus is now applied to the egg, × 670.

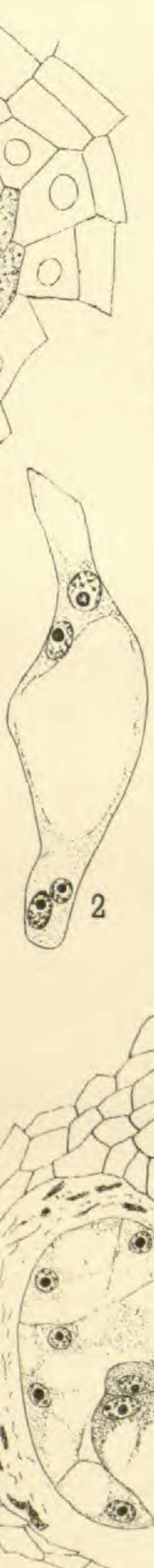
Fig. 8a. Egg and endosperm nuclei of the same, × 1350.

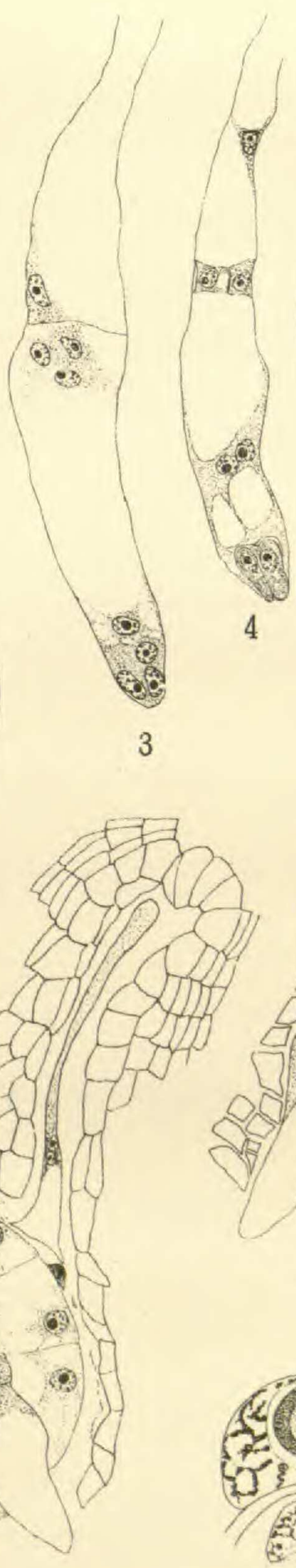
Fig. 9. After fertilization; embryo is still one-celled; several endosperm nuclei are now present; the disintegrated megaspores are now nearly absorbed; the endosperm begins to encroach on the integument, × 315.

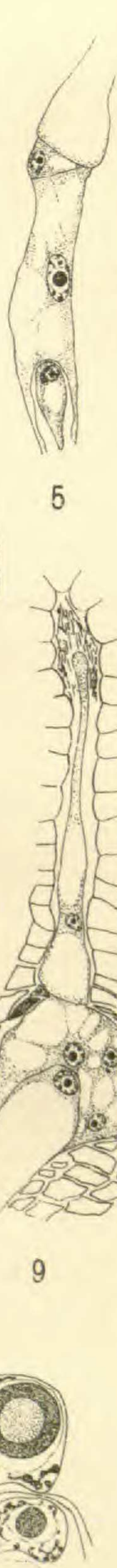
Fig. 10. Embryo two-celled. The endosperm, in which walls begin to appear, encroaches further upon the integument, × 315.

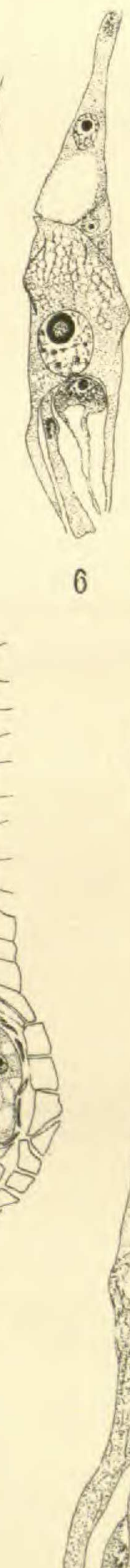
Fig. 11. Three antipodal cells, × 670.

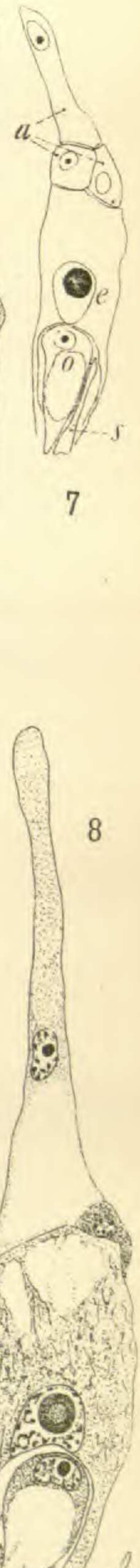


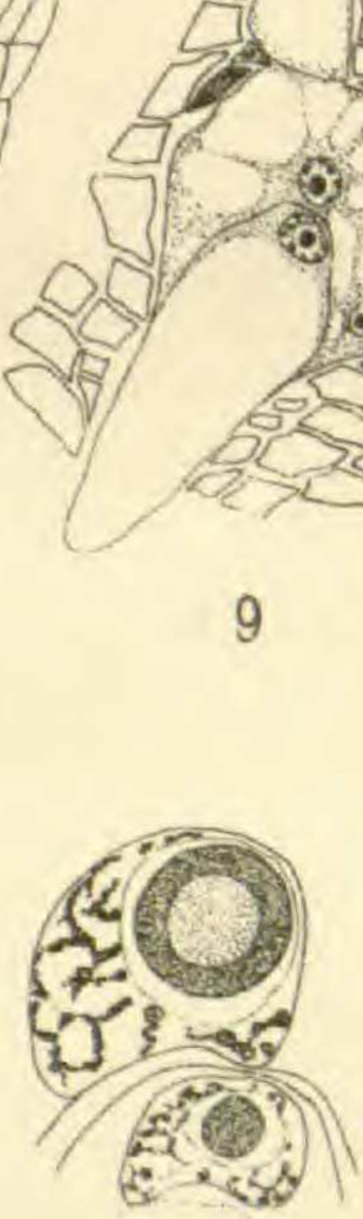












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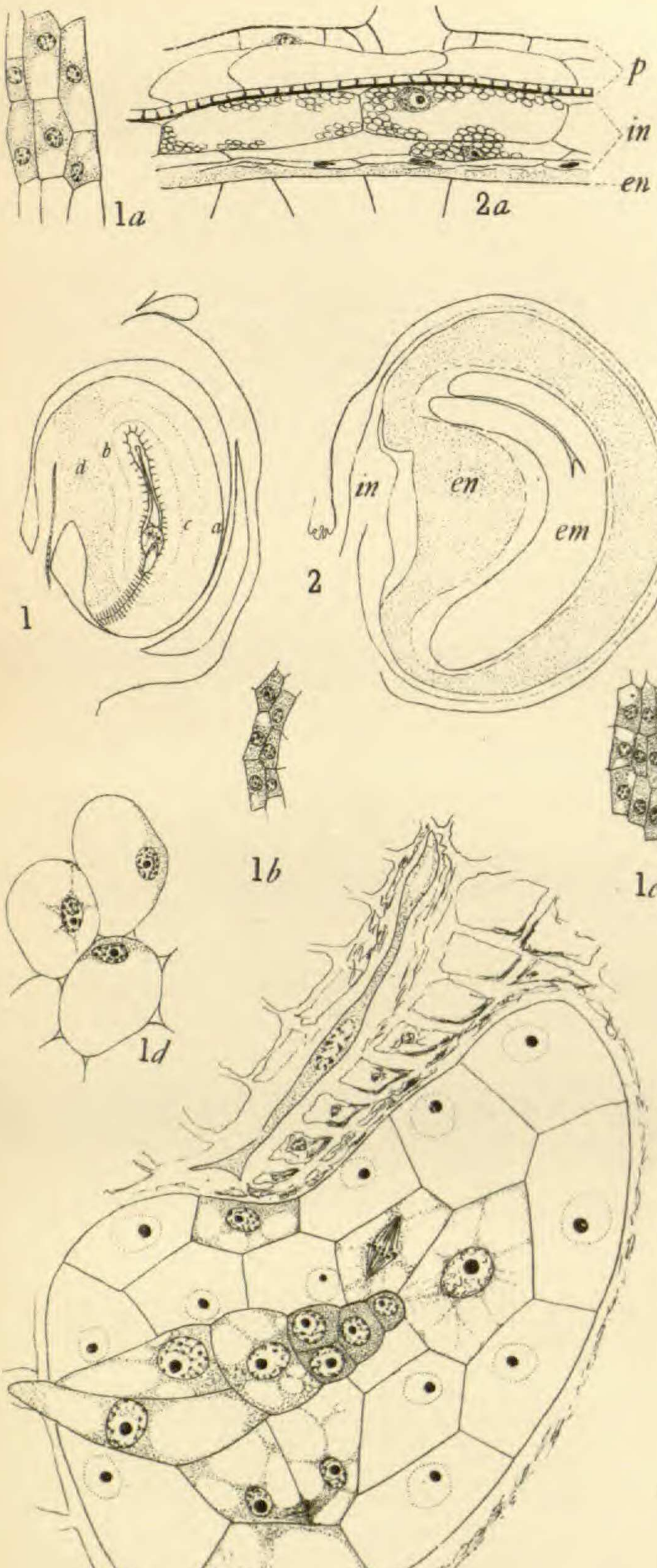

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Explanation of Plate 3

Fig. 1. Ovule just after fertilization showing areas made up of cells of different character. (*a*) Zone of cells dividing mostly by periclinal walls (see Fig. 1*a*). (*b*, *c*) Zone of rapidly multiplying cells (see Fig. 1*b*, 1*c*) forming a meristem completely surrounding the internal mass including the embryo-sac. (*d*) Mass of cells lying between the funicle and the meristem, made up of large cells (see Fig. 1*d*) containing vacuolated cytoplasm and starch grains, × 75.

Figs. 1*a*, *b*, *c* and *d*, × 670.

Fig. 2. Longitudinal section through mature fruit, in. integument, en. endosperm, em. embryo. The pericarp is seen as a thin layer without the integument.

Fig. 2*a*. Envelopes of mature fruit in detail. *p*. pericarp, *in*. integument, *en*. endosperm. The cells of the integument are still supplied with much starch, × 220.

Fig. 2*b*. Cells from periphery of endosperm on convex or dorsal aspect, × 472.

Fig. 2*c*. Cells from periphery of endosperm on concave or ventral aspect, × 472.

Fig. 3. After fertilization. The basal antipodal has its haustorial end embedded in a mass of refractive substance which results from the disintegrated megaspores. Embryo still one-celled, × 670.

Fig. 4. Young embryo lying in the endosperm. The latter by its growth pushes past the long antipodal into the integumental tissue as toward *c*, Fig. 1, × 472.

Explanation of Plate 4

Fig. 1. Proembryo of 5 cells, × 670.

Fig. 2., 3., 4. × 472 } Successively older stages of the embryo.

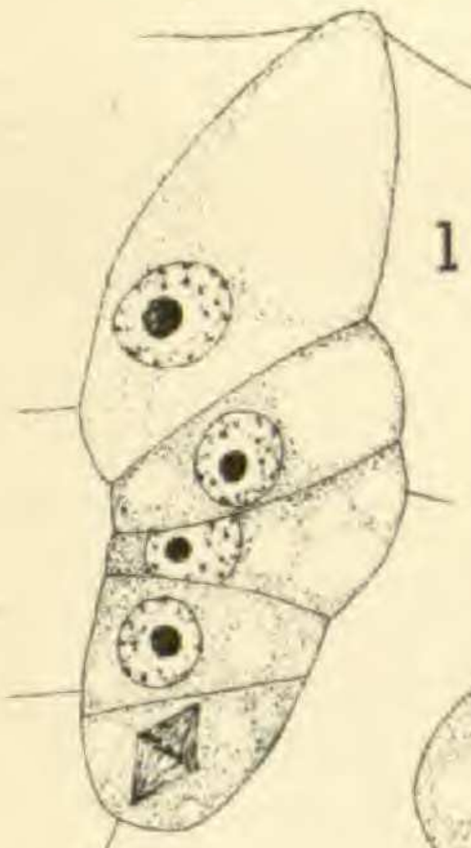
5., 6., 7. × 315 } Successively older stages of the embryo.

8. × 75 } Successively older stages of the embryo.

9. × 50 } Embryo, drawn on larger scale in Fig. 7, lying in the endosperm.

10. × 75 } " " " " " " " " 3, " " " " "

A comparison of these last two figures shows the position taken by the proembryo as a result of the growth of the endosperm at the micropylar region.



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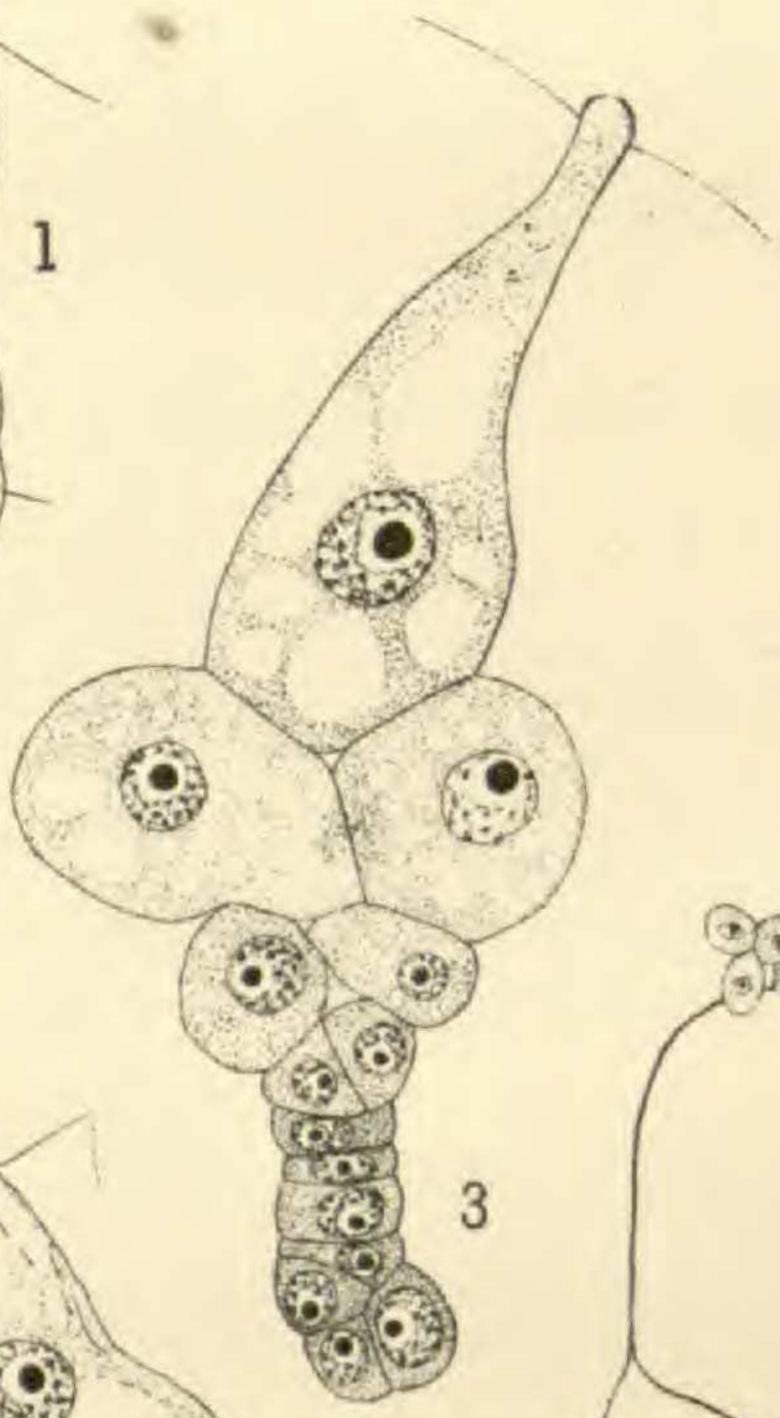

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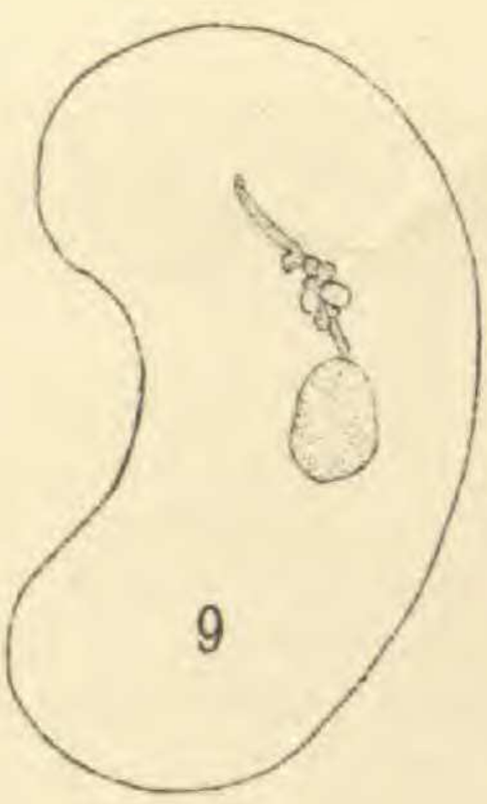

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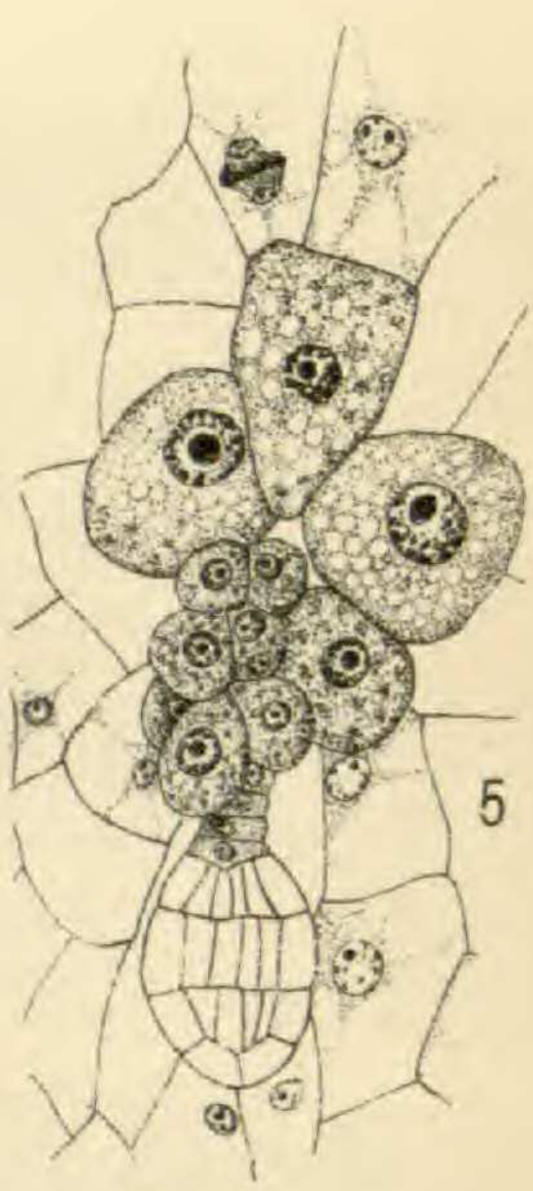

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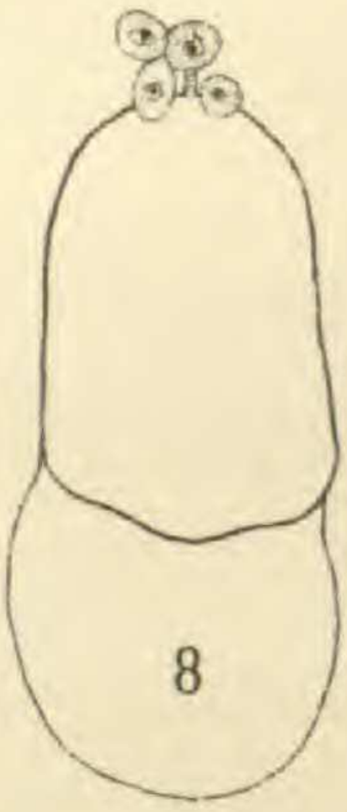

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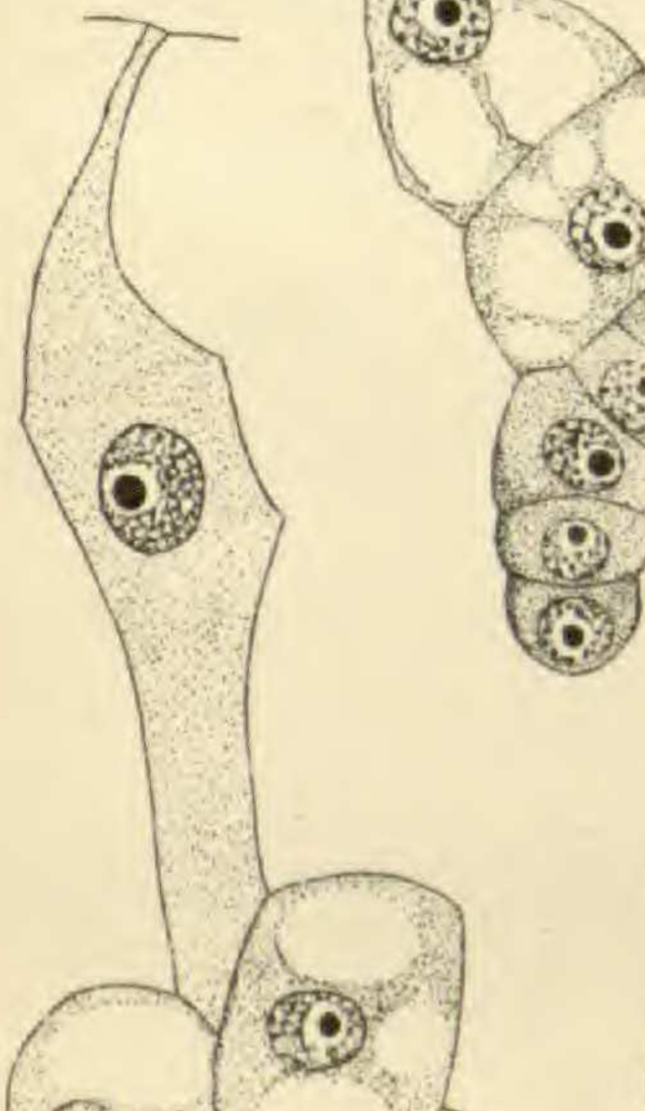

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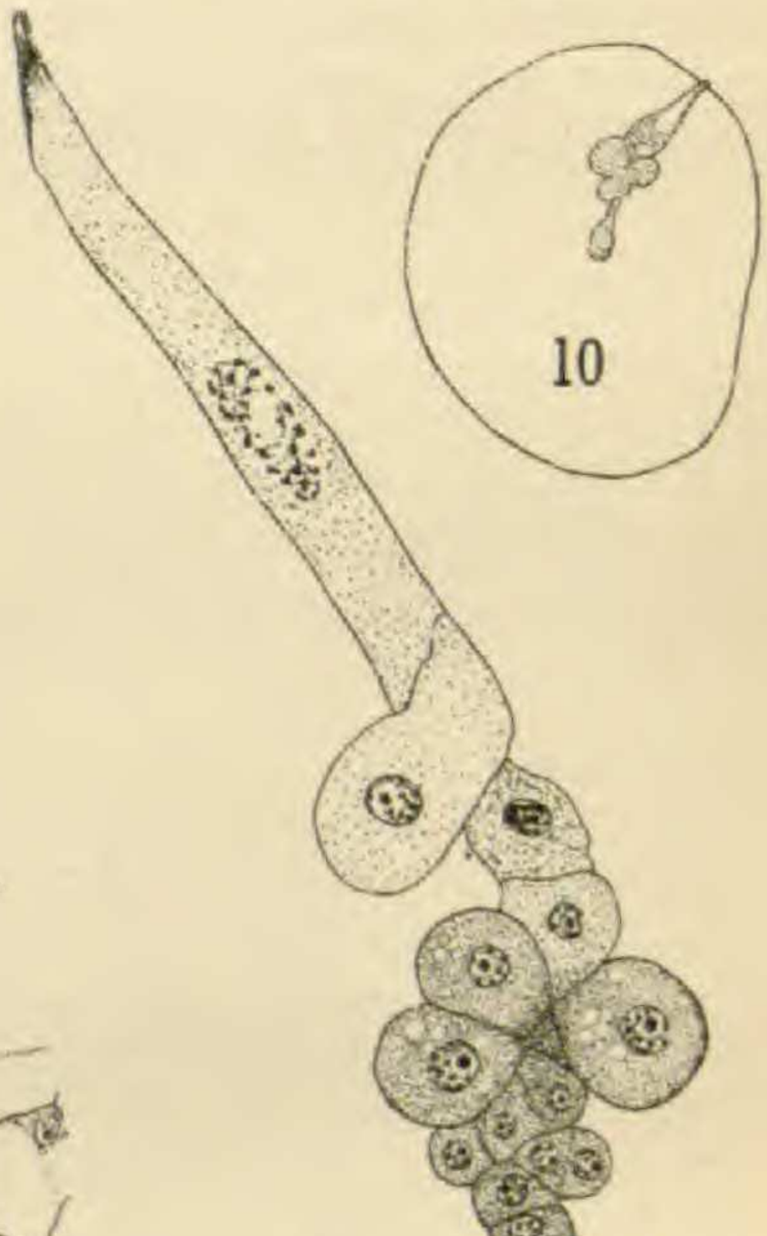

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Address, **L. M. UNDERWOOD**, Editor,
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VOL. VIII NO. 1, PART 2

THE COMPARATIVE EMBRYOLOGY

OF THE

RUBIACEAE

BY

FRANCIS ERNEST LLOYD

ISSUED FEBRUARY 15, 1902

FURTHER DESCRIPTION OF GENERA

Callipeltis cucullaria

The genus *Callipeltis* embraces three species indigenous to the Mediterranean region. In general habit they resemble closely the plants of the genus *Galium*, from which, however, they differ in their smaller size. *Callipeltis cucullaria*, the species studied, was collected in growing condition in the Botanical Garden at Berlin.

The Nucelli

The nucelli, two in number, arise as in *Vaillantia*, on either side the center of the floor of the ovary. Nor is their course of development different from that already described for that genus, excepting in matters of only minor moment, and these are to be found in the total number of cells in the nucellus, the greater relative amount of archesporial tissue, and in the shape of the definitive ovule.

The archesporium, the cells of which are more numerous than in *Vaillantia*, is evident when the nucellus is quite small (*pl. 5, fig. 1*). The epidermal capping cells remain without any further growth, while immediately around the area occupied by them, the integument grows out, as is made evident by the numerous periclinal divisions to be seen at this stage (*pl. 5, figs. 2 and 3*). By subsequent growth, the integument is developed so as to shut in a long micropylar canal (*pl. 5, fig. 4*). As the ovule attains its definitive form, the absence of the large special cells seen in the ovule of *Vaillantia* is noticed, and the sporogenous cells of the archesporium divide. The division into four megaspores is by no means complete for each cell. Some of them, the more centrally placed, divide more rapidly and completely, while the more peripheral lag behind and sometimes fail of division, or perhaps divide but once, and so a heterogeneous mass results, out of which the megaspore which develops into the embryo-sac makes its way into the micropylar canal. In such

a mixture of cells it is not easy to distinguish this cell, especially as it often happens that several pass through the initial stages of migration, and the result is very confusing. I have, however, with reasonable certainty, been able to pick out the most active cell, as in *figs. 4 and 5*. The embryo-sac cell, so far as determined, may arise out of any of the megaspores, so that we may regard these for the greater part as physiologically equivalent. The appearances presented by the archesporium at this time match closely those seen in *Alchemilla* as described by Murbeck.* Tapetal cells are not formed.

No starch is seen in the integument until the migration of the megaspore, when a slight accumulation occurs, evenly distributed throughout the whole. During the maturing of the embryo-sac, the amount of starch increases in a zone surrounding it. As the endosperm starts to grow, zonal accumulation disappears and a general distribution is again seen which becomes gradually more pronounced in the peripheral parts.

THE DEVELOPMENT OF THE EMBRYO-SAC

One of the megaspores which have developed as described above commences to enlarge and to migrate along the micropylar canal. This takes place when the ovule is well developed and has taken on its mature form. *Pl. 5, fig. 4*, shows these conditions just previous to the migration of the megaspores. It not infrequently happens in this species that two or even three megaspores commence to migrate at the same time and take paths which are nearly parallel. In such cases a good deal of confusion arises, which makes it difficult to interpret properly the preparations showing this condition. The remaining megaspores retain their character for some time, some degenerating more rapidly than others, and the resulting mass adds not a little to the difficulty of making out clearly what one sees. Occasionally two of the migrating megaspores germinate, although in no cases which I have observed has development passed beyond the four-celled stage (*pl. 5, fig. 8*) in any but the more advanced and normal one, which is set apart as the fundament of the functional embryo-sac.

* Murbeck, Sv. Parthenogenetische Embryobildung in der Gattung *Alchemilla*. Acta Reg. Soc. Physiogr. Lund, 11 : 1–47. 1901.

More than one embryo-sac may sometimes reach perfect development, thus supplying the conditions for false polyembryony. This, however, has not been observed.

The movement of the megaspore down the micropylar canal is accompanied by histolysis of the endodermis, the cells of which degenerate and collapse. The contents of the cells are disorganized and become deeply staining. It is to be noted that the nucleus is preceded in its course by a part of the cytoplasm, while the nucleus itself travels unusually near, or along the side of the cavity formed and some little distance back from its apex (*pl. 5, fig. 6*). The amount of cytoplasm is now not great, nor does it increase markedly in amount until the complement of embryo-sac nuclei is reached. The cells of the egg apparatus, as soon as they are cut off, become rapidly different in character from the rest in attaining greater density. Their final character is reached at about the time of fusion of the polar nuclei or somewhat later. The synergids then are vacuolated at their free ends, while the egg-cell has a vacuole at its narrow basal end. The fusion of the polar nuclei occurs near the middle of the endosperm cavity (*pl. 5, fig. 8*) and is followed by a movement of the resultant nucleus toward the egg-cell with which it lies in contact till its first division. Meanwhile the cytoplasm loses its vacuolated character and becomes more dense and gorged with food material, consisting, in large part, of starch. The whole endosperm-cell becomes more rounded also.

THE ANTIPODAL CELLS

When the fusion of the polar nuclei is taking place the antipodal nuclei have arranged themselves in their definitive positions, two of them close together, each with its proper mass of cytoplasm which is thin and vacuolated, and the third some distance away in the narrowing end of the somewhat irregular cavity formed by the megaspore in its migration (*pl. 5, fig. 9*). The cytoplasm of this latter antipodal cell is at first extremely scanty, so much so that it cannot be detected with a high power except near the nucleus. The two small antipodals secrete cell walls and remain in a normal condition for some time during the development of the embryo. Their cytoplasm becomes more dense from the time they are first

cut off, and numerous starch grains may then be seen. The long antipodal which occupies about three-fourths of the length of the entire embryo-sac takes on a definite club shape at its free extremity which reaches quite to the end of the megaspore cavity, and is finely granular, though not so densely filled with protoplasm as are the others. A large vacuole appears constantly at the thick end which abuts against the small antipodals. The vacuole extends as far as the nucleus. The interior of the narrow upper part is also occupied by a vacuole, though this is less evident in sections on account of the slender character of this region of the cell. A normal appearance is presented by this cell as by the other two until the proembryo has reached an eight- or ten-celled stage or even older (*pl. 6, fig. 5*), and an application of the iodine test demonstrates that at such a time the small antipodals are gorged with starch while the other shows a similar starch content at its thick end. The haustorial end stains deep yellow and is free from starch. No trace of the deeply staining products of disintegrated megaspores may be seen after the second or third division of the endosperm nuclei, though this is, of course, a somewhat variable matter. Disintegration of the antipodals sets in with the rapid extension of the endosperm, and they are finally lost to view.

ENDOSPERM AND INTEGUMENT

Soon after fertilization the endosperm nucleus divides. This division is followed by several others, probably five or six, before any walls are laid down. Its starch content is drawn upon during these divisions, so that at the time the cell walls are laid down, only a very little may be found or none at all. The proembryo remains unicellular till about the third or fourth division of the endosperm nuclei occurs. The growth of the endosperm is accompanied by destruction of the integument. The peripheral endosperm cells become less vacuolated, the greatest density of contents being found in those cells of the endosperm which lie towards the raphe, *i. e.*, the peripheral cells are most active where the largest amount of food is available. The accumulation of starch is gradual at first and is localized in the chalazal end of the endosperm. Later all the cells become gorged with starch, so that at the time the cotyledons appear, all the cells are well supplied with

the exception of those which occupy the middle portion of the endosperm and the layer of absorbing cells next the raphe. The endosperm continues however to grow, and ceases only when there is left of the integument only a single layer of cells separating the endosperm from the pericarp. The limit of growth in extent being reached, the cells commence to secrete reserve cellulose. Their lumina, by reason of the accumulating cellulose, become more contracted and rounded in form. The increase of cellulose is accompanied by an evident reduction of the starch content, though a plentiful supply is present at the maturity of the seed.

The immediate supply of food necessary for the endosperm is to be found in the integument, of which the starch content is gradually increased as it reaches its maximum size. When the seed is ripe, a single layer of integumental cells only is left, excepting an island of tissue underlying the termination of the vascular tissue of the funicle. The endosperm is thus a concavo-convex mass, surrounded by an integumental membrane, this, in turn, being closely invested by the pericarp four cells in thickness (*pl. 6, fig. 12*). A considerable amount of starch is found in all the cells of the integument, particularly in the chalazal island.

DEVELOPMENT OF THE EMBRYO

As already pointed out, the embryo remains unicellular till the endosperm nuclei have reached the number of eight to sixteen, when it begins to develop rapidly, one transverse division following another until the embryo is composed of a cell row of ten or a dozen cells (*pl. 6, fig. 6*). The largest of these are in the suspensor. The terminal cell, the fundament of the embryo proper, is the smallest. The suspensor cells bulge out more or less. A portion of the suspensor may be composed of two rows of cells (*pl. 6, fig. 9*), though, whether this is due to transverse division or to displacement by longitudinal pressure, I cannot say. Probably, however, the latter. The arrangement and number of cells in the suspensor is by no means constant, so that it is a matter of physiological significance alone. By further growth, the bulging suspensor cells extend themselves, so as to lie between the endosperm cells, but do not cause any destruction. They are filled with cytoplasm, and have large active nuclei. The development

of haustorial cells by the embryo in *Callipeltis* is not so great as in *Vaillantia*, either in point of number or size. With the development of the embryo proper and of the endosperm, the basal cell elongates, allowing the embryo to take a more central position in the endosperm. The haustorial portion is then near to the embryo. The basal cell is finally crushed between the cells of the endosperm, the haustorial portion becomes absorbed, so that only a single cell is left as a portion of the root cap of the embryo.

The development of the embryo is briefly as follows: After transverse division has ceased, the terminal-cell divides longitudinally once, then a second time. The second cell from the end follows suit, then the third and finally the fourth. Meanwhile the first three tiers of cells are giving rise to dermatogen, then to plerome in the fashion described by many authors for numerous dicotyledons. The fourth tier of cells which apparently never exceed four in number, remain so and may be recognized in the definitive embryo as a small protuberance at the top of the root cap.

A great deal of variation takes place, but the plan just set forth is followed in general and the ultimate product is the same.

Sherardia arvensis

This plant, the only species of the genus, is a small upright annual having the whorled foliage of the Galieae with small heads of several flowers with rose colored tubular corollas. It is distributed in Western Asia, Europe and North Africa. The material for study was obtained in cultivation in Germany and in New York.

The history and character of the nucelli, integument and megaspore mother-cells call for no special treatment. No evidence has been obtained pointing to differences in the division of the megaspore mother-cells, or in the selection of a given megaspore to become the embryo-sac. It thus appears that the megaspores are of potentially equivalent value. During migration down the micropylar canal the three serial divisions of the embryo-sac nuclei take place and finally a definitive embryo-sac is formed which differs somewhat from those hitherto described in form and

proportion (*pl. 6, fig. 15*). The two small antipodals are concavo-convex, and are set upon the endosperm cell as a cap. They are densely filled with food materials. The third antipodal is longer relatively than in either *Vaillantia* or *Callipeltis*, and is scantily supplied with cytoplasm. The distal end is formed into a club-shaped swelling, and is buried in the mass of disintegrated megaspores. The swollen end is filled with a tenuous and finally granular cytoplasm.

The endosperm nucleus lies in the definitive embryo-sac against the egg, into which position it has moved from a distant position where fusion of the polar nuclei takes place (*pl. 6, figs. 15, 16, 17*).

The development of the embryo proceeds by the formation of transverse walls, until about a dozen cells in linear arrangement have been laid down. All but the terminal cell form then the suspensor in which two distinct regions are to be recognized. The micropylar region is composed of larger vacuolated cells, which grow out laterally into the endosperm forcing their way between the cells. The nucleus lies in the concavity of the outgrowing haustoria just as is known to occur in young root hairs (*pl. 6, fig. 19*). The proximal portion of the suspensor is composed of very short disc-shaped cells, which, while the embryo is young, retain that form. As the embryo becomes older, the more distal of these elongate, and some grow out into haustoria. When the endosperm has reached its maximum size the laying down of reserve cellulose begins in the peripheral cells first, and these cells become unfavorable for the absorption of food by the suspensorial haustoria. That portion of the suspensor, therefore, which comes to lie in the region of cellulose formation degenerates *pari passu* with the thickening of the cellulose layer. The function of absorption thus given up by the more distal part of the suspensor is then taken up by the more newly formed haustoria. The suspensor becomes loaded with food which is gradually passed on to the embryo (*pl. 6, fig. 21*).

In the development of the embryo proper there is correspondence with the process as described for *Callipeltis*. One feature appears somewhat different, namely, that the longitudinal division of the terminal cell is followed by transverse division of its quad-

rants, so that it appears as if the terminal five cells had gone to form the embryo proper instead of four.

The mature ovule consists of a mass of endosperm with cellulose as the chief reserve food, and one layer of integumental cells still intact. The whole is closely invested by the pericarp.

The endosperm absorbs the integument till but one or two cell layers are left, these forming a membranous lining of the pericarp.

Galium

(PLATE 7)

The species of *Galium* which have been studied are the following: *G. Aparine*, *recurvum*, *pilosum*, *Mollugo*, *verum*, *triflorum*, *tinctorum*, and *Parisiense*. As the species of this genus are very similar with regard to the characters which we are considering, it is not deemed necessary to treat them separately.

The origin of the nucelli is in all respects as in the forms already described, and the same may be said of the number of cells in the archesporium (*figs. 1, 2 and 3*), their division into megaspores by two successive divisions (*fig. 4*), and the subsequent enlargement of one of these to act as the embryo-sac cell. In occasional instances two such cells derived from different megaspores may enlarge and when germination takes place, two more or less completely formed embryo-sacs result (*fig. 11*). In one case three such were found, although here the third embryo-sac was very abnormal in appearance. The nucellar epidermal capping cells remain intact until the megaspores are fully formed, but suffer histolysis at about the time the migration of the embryo-sac mother-cell commences (*fig. 4*). The histolysis of the endodermis then follows, as the enlarged megaspore moves forward along the micropylar canal.

The condition of the definitive embryo-sac is reached in the usual manner. A two-celled condition is shown in *fig. 6*. The chief interest in this connection attaches to the form the embryo-sac takes in the various species of the genus under consideration. The various degrees of curvature seen in different species is, no doubt, due to the form of the ovule. The more strictly anatropous the ovule, the straighter the embryo-sac. In *Galium tinctorum* in which the ovule approaches the campylotrous condition, the embryo-sac is bent into an arc of ninety degrees (*fig. 10*). In other

respects save in three particulars the characters of the embryo-sac are uniform namely, in the form of the endosperm cell, the shape and disposition of the antipodals and the amount of the food content. In certain species, *e. g.*, *G. Mollugo*, and *G. Parisiense*, the endosperm cell is almost spherical while in others it is oval. In *G. tinctorum* this cell is markedly constricted about midway its length and this appears to be the normal condition (*fig. 10*). In this plant also, the food content of the endosperm cell is evidently greater, and is composed for the most part of starch. The starch grains are indeed so crowded that the nucleus is very much distorted by compression (*fig. 10*). Finally the antipodal cells may take two different arrangements. They may be placed tandem fashion as in *G. triflorum* (*fig. 7*) and *G. Mollugo* (*fig. 11*), or the two small antipodals may lie in the same transverse plane as occurs in *G. Aparine* (*fig. 8*) and *G. Parisiense* (*fig. 12*), and some others. A modification of this latter mode of arrangement is to be found in *G. tinctorum* (*fig. 10*), in which plant these cells become rounded and are placed obliquely with reference to the longitudinal axis of the embryo-sac. These cells are at the same time densely filled with starch, a good deal of which material is also to be found in the long antipodal cell.

The haustorial antipodal cell reaches, in this genus, its greatest relative length, as is illustrated by our *fig. 9* (*G. triflorum*), which shows an ovule in longitudinal section, with an embryo-sac just before fertilization.

The development of the embryo and endosperm in their earlier stages presents no differences of special note. Considerable variation is shown in the development of the suspensor in the different species. In *G. Parisiense*, for example, there is but a small haustorial apparatus formed, while the suspensor of this plant is remarkable for the unusual elongation of the basal cell (*fig. 13*). Of the species studied, the greatest development of the suspensor is reached in *G. Mollugo* (*fig. 15*).

The endosperm arises by division of the endosperm nucleus in all directions, at first without the formation of cell walls. Soon, however, these commence to form before, indeed, the suspensor produces haustoria. The histolytic changes in the integument, which take place relatively slowly at first, proceed a little later

very rapidly, and, as indicated in *fig. 14*, the histolysis is not confined to the immediate vicinity of the endosperm. In the same figure we may note the antipodal cells to be still present, but they are in a dead condition, and it would not appear that they are in any way active agents in the action described, but rather that the enzyme which we may believe is secreted by the endosperm more readily passes along the course of the antipodal apparatus and is thus distributed to a distance from the endosperm.

That the behavior of the integument as the endosperm grows is indeed the result of the action of an enzyme, we have evidence in the case shown in *fig. 15*, where the cell-walls of the cells lining the micropyle remain unaffected, although the surrounding tissue has been totally destroyed.

It is to be noted also that the endosperm nucleus takes its position against the egg cell, although this does not appear to be the case in *G. tinctorum*. This may be due to the interference of the large and numerous starch grains which crowd the cell and would very naturally interfere with the movements of the endosperm nucleus (*fig. 10*).

The food mass derived from the disintegrating megaspores becomes exhausted at the maturity of the embryo-sac or soon after and no trace of this may be seen soon after fertilization. At this time, too, the fertilized egg loses its vacuole by the increase of the cytoplasm, and the synergids at the same time disintegrate.

The course of development of the embryo is, in the early stages, like that in the others previously described.

Asperula

(PLATES 8 AND 9)

Of this genus the following five species have been studied: *A. azurea*, *galioides*, *montana*, *setosa*, and *tinctoria*.

The development of the ovule and the appearances presented by the archesporium in the genus *Asperula* do not differ in general from the other *Galieae*. They present, nevertheless, some striking points of contrast, especially when compared with *Crucianella*.

The early stages of development of the ovule may be passed over without further remark. When the definitive condition of the archesporium has been attained, the further development of

the ovule, especially of the integument, is such that the upper part of the sporogenous tissue becomes compressed so that the individual cells become displaced and distorted to such a degree that the whole appears almost abnormal. This appearance is accentuated during and after the division of the mother-cells, which, it would seem, is by no means as regular as in *Cruciancilla*. This irregularity is most striking in *Asperula montana*, in the ovule of which plant the compression appears to be greatest on the micropylar part of the archesporium. This condition is obviously the cause also of certain curious abnormalities in the behavior of the embryo-sac to be described shortly. The irregularities in the behavior of the archesporial tissue consist in the differences in rapidity with which tetrad formation takes place, in the partial or total suppression of tetrad formation in a greater proportion of the megaspores, in the early destruction, by pressure, of some of the cells, and finally are such that the accurate observation of the behavior of individual cells is rendered almost impossible.

That tetrad formation takes place normally, however, is quite sure, from the fact that, in spite of the confusion, rows of four cells each are repeatedly found. The megaspores, sister to the functional embryo-sac cell, persist in apparently normal condition for a period at least equal to that occupied by the development of the embryo-sac, and occupy, forasmuch as they do not secrete cell walls, the same cavity as the embryo-sac. It sometimes happens, too, that they have quite the appearance of the antipodal cells, so that it becomes difficult to distinguish between them.

After the functional embryo-sac cell has started to migrate (*pl. 8, fig. 2*), some of the other megaspores persist, while others degenerate. Some, further, secrete cell walls, and undergo changes which indicate that they are passing through early stages of embryo-sac development. One such cell is shown in the upper right-hand part of the figure.

It seems equally certain that the tendency is for the upper of the tetrads to become the embryo-sac. The pressure already referred to, however, modifies the ordinary mode of procedure, so that not only may some other of the megaspores rather than the upper become the embryo-sac, but it may also be forced to develop in quite the wrong direction. This is the case in *Asperula mon-*

tana, in which a completely developed embryo-sac is frequently to be found in the chalazal part of the ovule, or even in the funicle. *Text figs. 1 and 2* illustrate two such instances. In *fig. 1* two

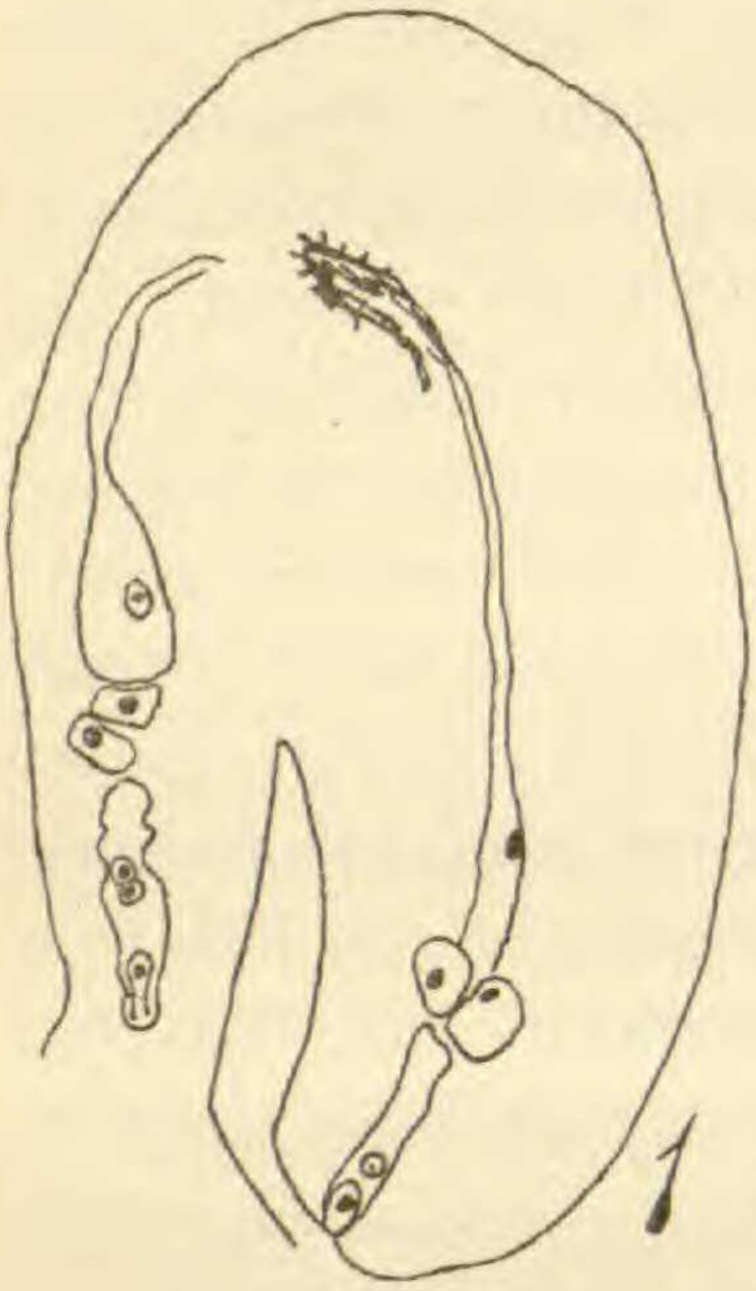

FIG. 1. Ovule with a second embryo-sac lying in the funicle.

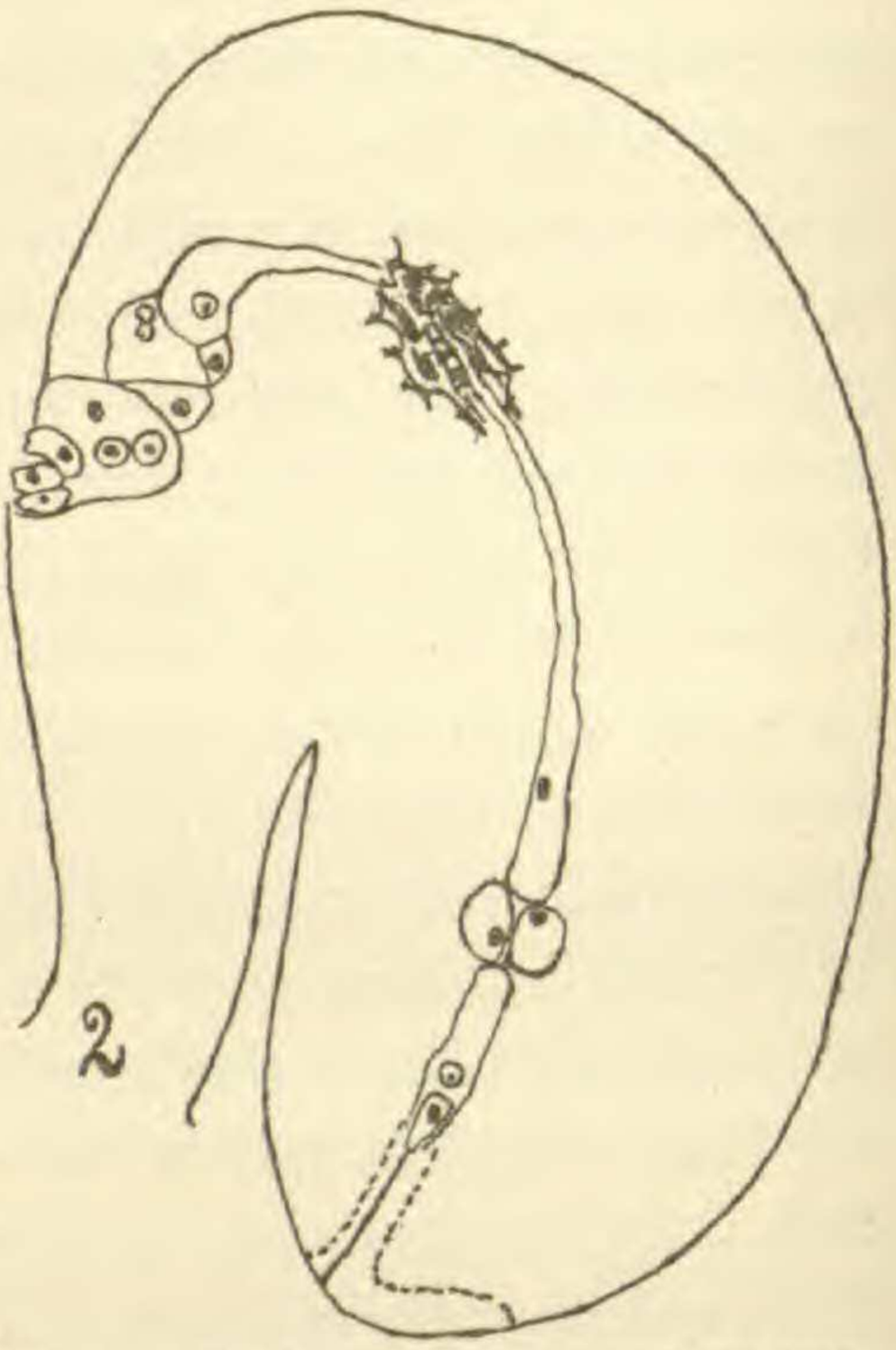

FIG. 2. Ovule with three embryo-sacs, two in the funicle and one in the usual position. The dotted line indicates the frequent protruding position of the embryo-sac.

embryo-sacs are seen; one in the normal position, *i. e.*, in the micropylar canal, and one lying parallel to it, but in the funicle. Both are completely developed. In *text fig. 2* three embryo-sacs may be seen; the one normal in position, and the two others lying against each other, in the funicle, and considerably distorted. The egg poles of these two embryo-sacs are placed against the pericarp; that is to say, the embryo-sacs have grown to the surface of the ovule. In *text fig. 3* the egg apparatus and polar nuclei of one of these abnormally placed embryo-sacs may be seen to be quite normal except for a small amount of distortion. Whether the egg of such abnormally placed embryo-sacs is ever fertilized is doubtful, and no case has been observed. This plant has flowers which show a peculiar form of dimorphism caused by the abortion of the ovules in a large percentage of cases.

Only relatively very few ovaries with normal ovules therefore are to be found, and from these only few seeds were produced under the cultural conditions at Bonn, where the material was collected. In view of these facts, therefore, the failure to observe fertilization in the abnormally placed embryo-sacs is not surprising. On the other hand, there are grounds for expectation that the behavior of the pollen tubes is such as to favor such an occurrence.

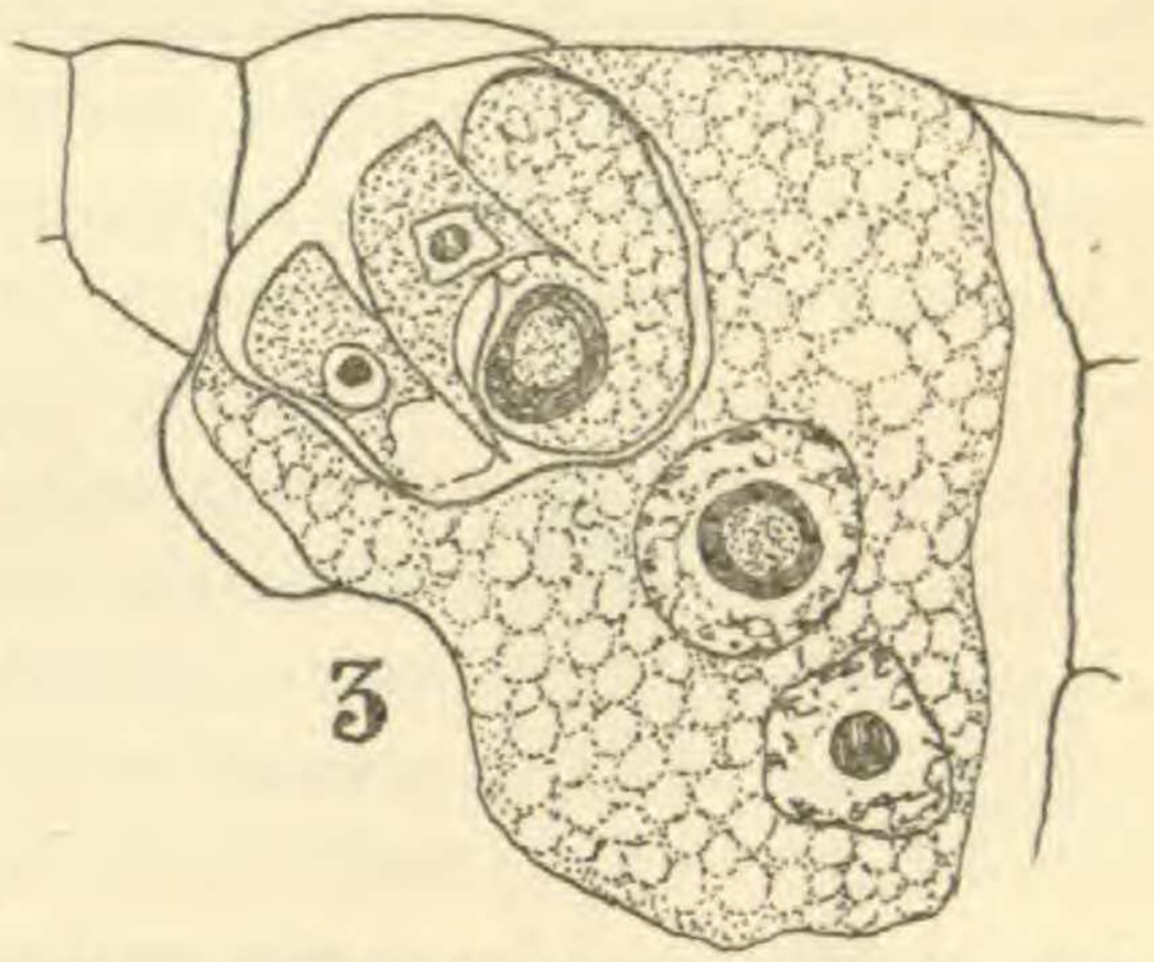

FIG. 3. Part of embryo-sac occurring in the funicle showing egg apparatus.

Further peculiarities occur in the behavior of the normally placed embryo-sac in *Asperula montana*, in that its growth continues often until the egg end projects beyond the mouth of the

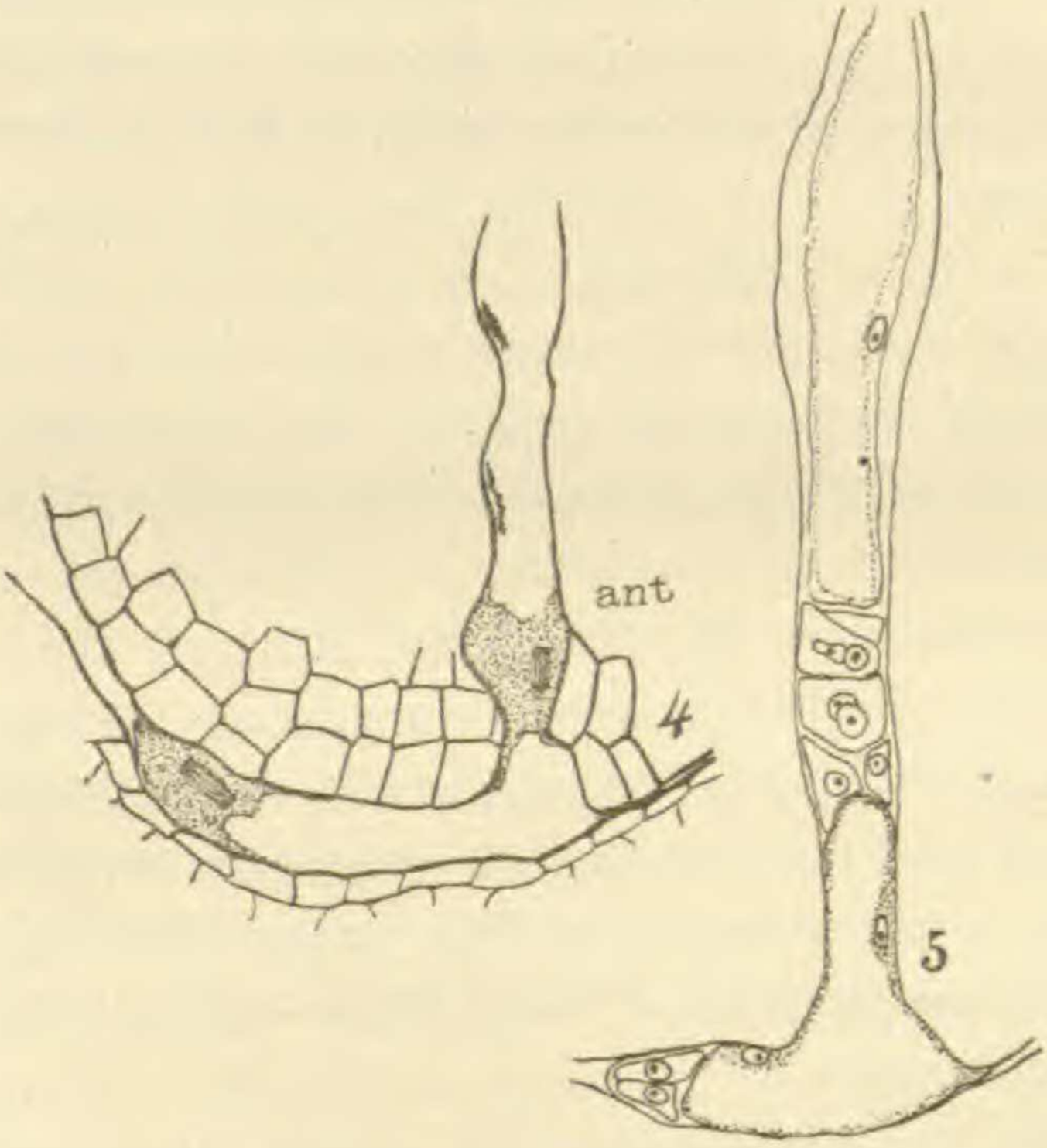

FIG. 4. Protruding embryo-sac with two nuclei in mitosis. *Ant.*, Antipodal nucleus.

FIG. 5. Protruding embryo-sac with at least seven antipodal nuclei. Polar nuclei not yet fused.

micropyle and comes to lie between the integument and the pericarp. In *text fig. 2* the dotted line indicates in a schematic way the position taken by the embryo-sac under these circumstances, and *text figs. 4 and 5* show two such embryo-sacs in detail. It is to be noted that the egg apparatus under these circumstances is always directed away from the funicle. In cases in which fertilization takes place, the egg elongates before division, and the embryo thus comes to lie within the ovule (*text fig. 6*). *Fig. 7* serves as an example, although here the egg apparatus is not as far away from the micropyle as in many other cases. The habit of the embryo of producing a long slender stalk before division is constant, and may be seen even where the embryo-sac does not protrude from the micropyle, where, namely, the embryo-sac takes the same position as in the other *Galieae* (*text fig. 7*). What the behavior of the pollen tube in this plant may be cannot be said, as no favorable material has so far been found. A study of this point cannot fail to be of interest.

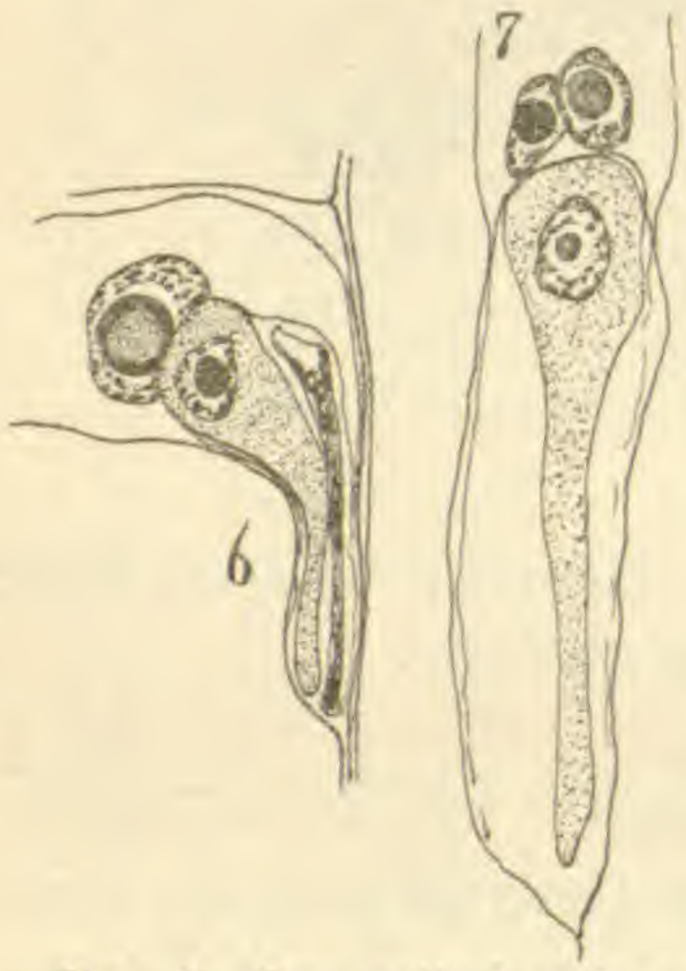

FIG. 6. One-celled embryo formed in a protruding embryo-sac.

FIG. 7. One-celled embryo in embryo-sac occurring in the more usual position.

The migration of supernumerary embryo-sacs into the funicle as above described is of special interest as it bears on the question of the guidance of the embryo-sac in its movements.

Under ordinary circumstances the embryo-sac cell migrates down the micropylar canal, breaking through the thin cap o nucellar tissue. Where, however, the pressure of the integument is great enough the normal movements of the embryo-sac nucleus are prohibited, and the embryo-sac makes an irregular growth in any direction, and reaches no definite point. We may, there ore, conclude that the guidance of the embryo-sac into the micropylar canal is mechanical.

The other species of *Asperula* studied show in general the same disturbances in the archesporium due to pressure as in *Asperula montana*, though not to the same degree. This is indicated by the fact that no exception to the normal position of the

embryo-sac has been seen, although similar appearances in the archesporial tissue are there.

The only other point in connection with the embryo-sac calling for special mention is the behavior of the antipodal cells. These are by no means constant in number, and may vary even in the same species, as far as observed, from three to seven. The most typical condition is shown in *pl. 8, fig. 3*, for *Asperula azurea*, and in *pl. 9, figs. 1 and 2*, for *Asperula montana*. The long antipodal has the usual appearance corresponding as it does to the homologous cell in the other *Galieae*. The other two, however, are here very much enlarged and each consists of dense cytoplasm with a large active nucleus, and a very large central vacuole. In the growth of these cells the adjacent tissues present histolytic appearances similar to those seen in the same tissue when the endosperm enlarges. There is, therefore, so much evidence that the antipodal cells are able to secrete substance which acts in the way indicated upon the living cells.

When more than three antipodal cells occur they are arranged in pairs forming an irregular double row, recalling the condition in *Aster* (Chamberlain) and in *Diodia* (*pl. 9, fig. 5*). In some species the behavior of these cells is very irregular; one frequently finds several nuclei in the same cell, and the cells are sometimes much more slender in proportion than as represented in the figure (*pl. 9, fig. 5*). In all cases, however, the same observations already made with reference to the persistance of the antipodals and their nutritive relations holds good in *Asperula*.

THE EMBRYO

Of all the *Galieae* studied, the genus *Asperula* is most remarkable for the extreme development of the suspensor. In *A. setosa* (*pl. 8, fig. 4*) the haustorial cells attain a large size, and extend to a considerable distance from the suspensor into the endosperm. The amount of cytoplasm is not great, and is collected mainly at the distal ends of the haustoria, where the nucleus is to be found. At this point the growth of the organ takes place, so that we have here a very close analogy with the root hair in which, as Haberlandt * has shown, the nucleus and cytoplasm behave similarly.

* Ueber die Beziehung zwischen Function und Lage des Zellkernes bei den Pflanzen, Jena, 1887.

In this form, on account of the large size attained by the haustoria it is quite easy to see that, as they penetrate the endosperm, the process is accompanied by mechanical readjustment of the cells of the latter, without any destruction. *Pl. 8, fig. 5*, shows a later stage in the same or a closely related species, from which it will be seen that as the embryo grows older the haustoria becomes filled with cytoplasm. Meanwhile their proximal extremities become compressed and drawn out as a result of the pressure exerted on them by the growing endosperm. The suspensor, exclusive of the haustoria, then presents a fibrous appearance, with little cytoplasm, and is little more than an irregular tube joining the bases of the haustoria and the embryo. The haustoria in some instances are very narrow, and extend to a considerable distance, passing beyond the embryo proper. Such narrow ones remind one of fungal hyphae, which pursue an intercellular growth, or of the pollen tube with its analogous behavior.

In the other species, for example *A. azurea* and *A. galioides*, the suspensor develops haustoria which, instead of penetrating to a distance into the endosperm, confine themselves to rather narrow limits, while the immediately surrounding endosperm suffers histolysis. All the preparations of plants in which this behavior takes place show a perfectly normal structure in the haustoria, in which the cytoplasm is very dense and finely granular as in *A. galioides*, while in the material in which they are embedded no trace of structure is visible, nothing, in short, but the mucilaginous irregularly staining products of histolysis.

As the embryo becomes older the haustoria become separated from each other but still keep a normal appearance. The study of a complete series, of which *pl. 8, fig. 6*, represents a component of three sections, shows no union between several of the haustoria, although this fact cannot well be shown in a drawing.

The behavior of the suspensor in the various species of *Asperula* is therefore so different, as well as the relation between it and the endosperm, that we are prevented from interpreting the facts in the same way. In some cases, as described, the relation involves histolysis of the endosperm, which indicates that, in such cases, the suspensor secretes an enzyme.

The earlier cell divisions of the embryo proper are as described

in *Callipeltis*. It is to be observed, however, that the longitudinal divisions which are generally confined to the last four cells of the young embryo here take place also in a half dozen or more of the cells of the suspensor nearest the embryo (*pl. 8, figs. 4 and 5*).

Rubia tinctoria

(PLATE 9, FIGURES 1–6)

The nucelli of *Rubia* are recognizable by their form which in the young condition, before the integument has commenced to grow, is characterized by a sharp bend on the inner side of the funicle. The megaspore mother-cells are fewer in number and are of different proportions, being shorter and thicker than the same structures in the rest of the *Galieae* studied. Their nuclei are relatively larger (*fig. 1*). The tapetal cells fail here as elsewhere, and the division of the spore mother-cells is regular (*fig. 2*) giving rise to four equivalent spores. It is a common occurrence for several of the megaspores to divide with the result that a confusion is caused. The migration of the proper embryo-sac cell along the micropylar canal is accompanied by the usual divisions and the ultimate formation of an embryo-sac (*fig. 5*). The haustorial antipodal cell is broader and shorter than those of the foregoing forms. The other antipodal cells may be very small, and placed laterally or in some cases are quite large. In the former case they are filled with cytoplasm, in the latter case they have a cytoplasmic lining and the interior is filled by a large vacuole.

It is interesting to note that the embryo-sac nuclei do not cause the disintegration of the endodermis so rapidly as in other *Galieae*, and appear to force their way along the micropylar canal, without the immediate collapse of the impinging cells (*fig. 3*).

In many respects the features of the archesporium and of the embryo-sac present the same peculiarities as have already been described in *Asperula*. There is the same tendency towards the formation of supernumerary embryo-sacs, and the same characters are shown by the persisting megaspores (*fig. 4*), the nuclei of which are supplied with a scanty amount of cytoplasm, and are found lying freely in the irregular cavities left by the megaspore mother-cells.

After fertilization the endosperm grows very rapidly, and gets a large bulk. Although the growth of the embryo is immediate, and as rapid at least as in the other plants so far described, it is proportionately slower, so that in a fruit so large that one would naturally expect to find a large embryo, one finds, on the contrary, a young embryo without cotyledons. The endosperm has large cells and contains a very small proportion of plasma. For this reason the endosperm plasmolyzes very easily.

The suspensor of this form is very fully developed (*fig. 6*). The cell divisions are, at first, solely transverse. Soon, however, they become irregular and often strictly longitudinal, while all of the suspensor cells excepting a few disc-shaped cells near the embryo proper, and still fewer at the micropylar end of the suspensor develop into haustoria. The relatively greatest development is reached by the suspensor when the embryo proper has about eight to sixteen cells. The pressure exerted by the endosperm upon the suspensor, together with the direction of growth of the same causes the suspensor to be drawn out, so that the embryo proper appears to be anchored to the endosperm, which in this way is enabled to exert a tension upon the suspensor.

Crucianella

(PLATE 10, FIGURES 7–22)

Of the genus *Crucianella*, three species, namely, *C. gilanica*, *C. macrostachya*, and *C. herbacea* have been studied. These present certain anomalies which are of not a little interest in view of the close relationship between *Crucianella* and the rest of the *Galieae*.

The archesporium consists of 12 to 15 very large embryo-sac-mother-cells, which, however, vary between themselves in size. The largest occupy the more central position, and these are also distinguished by the completeness of their nuclear divisions; the other cells divide less regularly or not at all, and the more quickly show signs of degeneration. The cell walls between the mother-cells are very delicate, but are present and easily distinguishable.

The division of the megaspore mother-cells each into four grand-daughter cells is remarkably regular, and results in the formation of four megaspores which are alike in size, but not separated by cell walls. This feature, to which only very occa-

sional exceptions may be found, may be regarded from the phylogenetic point of view as a step further removed from the condition in Angiosperms in general in which the tendency to form cell walls may be regarded as an inheritance from forms in which the megaspores are in maturity free bodies.

Similar failure on the part of the megaspores to form cell walls has been reported to occur in *Eichhornia* * and in *Avena* † and is probably of much wider occurrence than at present supposed.

Certain authors—Juel, Murbeck and Koernicke especially—have recently concentrated attention anew to the constancy and significance of these divisions—the tetrad division. In addition to the evidence adduced from the character of the nuclear mitoses, to which reference is made in another part of this paper, the facts which speak for the morphological equivalency of the cells which justifies the use of the term megaspores as applied to them have been set forth at length by Koernicke.‡

Remarkable as is the regularity with which the megaspore mother-cell divisions take place, still more so is the further division, in very many instances, of *all the megaspores simultaneously*. If we regard these as the first embryo-sac divisions, which we are justified in doing from a comparative standpoint, we are forced to regard the eight resultant nuclei derived from a single megaspore mother-cell as representing four embryo-sacs lying tandem. It has been determined by actual count that some ovules may thus be supplied with a dozen young embryo-sacs. Of these two or three of those derived from the megaspores lying adjacent to the micropylar canal may commence to develop into functional embryo-sacs. Only one, however, normally attains full development. The very evident physiological as well as morphological equivalency of the megaspores in this plant irresistibly compels the comparison with the conditions seen in certain heterosporous vascular cryptogams, especially with *Selaginella*, in which the majority of the megaspores serve as nutriment for the functional ones.

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* Smith, W. R. A Contribution to the Life History of the Pontederiaceae. Bot. Gaz. 15: 1898.

† Cannon, W. A. A Morphological Study of the Flower and Embryo of the Wild Oat, *Avena fatua* L. Proc. Cal. Acad. Botany, III. 1: 329. 1900.

‡ Studien an Embryosack-Mutterzellen. Sitzungsber. Gesellsch. Natur. und Heilk. Bonn, 1901.

The occurrence of such conditions in widely separated families is, however, a fact derived from comparative study, which warns us from inferring phylogenetic continuity. We are dealing here with *recurrent morphological structures*, called out in response to physiological necessities.

Of the four megaspores formed, although as just pointed out their activity is indicated by their tendency to further division, only the upper one, namely, the one furthest removed from the chalaza, develops into a functional embryo-sac. No exception to this rule has been observed. Instead, also, of enlarging directly at the expense of the other three by destroying and absorbing them, it moves into the micropylar canal, meanwhile growing at the expense of the adjacent tissue of the ovule (*figs. 18, 19, 21, 22*). In so doing the nucellar capping cells are destroyed. The fate of the remaining megaspores of the tetrad is indicated in *fig. 19*, in which they may be seen to have undergone partial disintegration, and to have been drawn forward toward the embryo-sac as if by suction. This appearance is quite constant, and is difficult to interpret in any other way. That the materials derived from them by their gradual disintegration and that of the other megaspores are used by the embryo-sac is shown by their gradual disappearance inversely to the growth of the embryo-sac.

For some time during the development of the embryo-sac, however, the tetrad masses maintain their normal appearance, and indeed elongate considerably, but generally show signs of degeneration before it is completely developed.

At the time the embryo-sac cell commences its migration down the micropylar canal certain changes take place in inner layers of the adjacent integument These consist in the rapid growth of the cells to many times their original volume, and the pronounced enlargement of the nuclei (*fig. 13*). These cells appear to be very active, and in some cases they fail to secrete cell walls, so that we find occasional cells provided with several nuclei. This behavior of cells, namely, their failure to form cell walls immediately, appears to be connected with a highly nutritive function, and recalls the conditions in young endosperm, in the suspensor of some *Leguminosae* (Guignard, *l. c.*), and in the megaspore masses described above. Examination shows that this cell mass

is a specialized portion of the ovule which is especially concerned in secreting starch, of which a very large amount is present, while it is at the same time absent from the rest of the ovule.

At the base of the archesporium the cells of the chalazal tissue appear, in a very early stage, just as the remainder of the cells of the ovule. As the integument develops, however, and therefore, as the archesporial cells increase in size, these chalazal cells take on a different character (*c.c., figs. 7, 9 and 13*). From being small cells with a rather scant supply of cytoplasm, and relatively small nuclei, they become larger, their nuclei grow, and they become densely filled with cytoplasm. This appearance they maintain during the periods of growth of the archesporial cells, of their division into megaspores, and of the subsequent elongation of the tetrad masses. At the close of the activity of the latter the chalazal cells lose their supply of cytoplasm and return to the condition of the rest of the cells of the ovule.

We may therefore interpret them as physiologically active in the nutrition of the archesporium. Similar specialized chalazal cells have been described in many forms, more recently, however, in *Polypompholyx* * and in *Stylidium.*†

The embryo-sac cell moves rapidly along the micropylar canal, and as it moves forward the integumental tissue closes behind it. The course taken may readily be traced by the more deeply staining cell walls of those cells lying next to the canal. The division of the embryo-sac cell follows the usual order, as heretofore described.

In this genus, however, the development of the embryo-sac is marked by the very sudden disappearance of the antipodal cells. Of all the preparations made, in not one have all three of the antipodals been seen, although they are developed, as indicated in *fig. 21, ant.* The polar nuclei have been seen, and several cases have been found where one antipodal nucleus appeared normal. All the evidence indicates, therefore, that the sudden death of these nuclei is a character of the genus. It must however be noted that the antipodal end of the embryo-sac extends some

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*Lang, F. K. Untersuchungen über Morphologie, Anatomie, und Samenentwickelung von *Polypompholyx* und *Biblis gigantea*. Flora, 88 : 149–206. 1901.

†Burns, G. P. Beiträge zur Kenntniss der Stilidiaceen., Flora, 87 : 313–354. 1900.

distance backwards toward the archesporial tissue, and is in connection with the same by means of the micropylar canal.

The embryo and endosperm present no points of striking difference from the same structures already described in other *Galieae*.

Diodia Virginiana

(PLATE 12, FIGURES 1–12)

I am indebted to Professor Frank S. Earle for collecting and preserving material of this and the following species described.

NUCELLUS. STROPHIOLE AND INTEGUMENT

From the account already given of the origin in the *Galieae* of the nuclei and basal partition, we have seen that the former rise independently from the floor of the ovary, and that the latter even at maturity of the ovules reaches over to one-fourth the distance from the floor to the roof of the ovarial chamber.

In the forms now to be considered, however, a somewhat different condition is to be found. Here the basal partition originates at the same time with the nucelli, so that in an appropriate longitudinal section one sees a plate of tissue rising from the floor of the ovary, from the sides of which the nucelli, seen as hemispherical masses, take their origin (*fig. 1*). With the further development of the ovary, the basal partition and that formed by the upper parts of the carpels, grow with equal rapidity, so that at the time the embryo-sac is mature the floor and roof moities of the ovarial partition fuse at a point half way from the floor to the roof of the ovary. At this point, therefore, are inserted the funicles of the ovules, whose growth is such as to give rise to the amphitropous form (*figs. 2, 8, and 9*).

Early in the development of the ovule at the time when, in assuming the amphitropous position, its axis is passing through a transverse plane, a growth which superficially suggests an integument makes its appearance first on the morphologically lower sides of the funicle. Coincident with the origin of this organ which for our present purposes we call a strophiole, the single integument commences to develop as a ring-shaped fundament about the epidermal capping cells of the archesporium. The growth of the integument is such as to form a long micropylar canal, the mouth

of which is ultimately covered by the strophiole. Certain features of special interest attach to this organ which will now be described.

In the first place, the supply of vascular tissue to the ovule is confined to the strophiole. A single strand of the same passes through the funicle and upon reaching the strophiole divides, sending one branch, the chief one, to the chalazal region of the strophiole, the other toward the micropylar region (*a* and *a'*, *figs. 2, 8, 9*). The chalazal branch (*a*) presents at its extremity a sharp turn towards the spot at which lies the archesporium.

Secondly the limit of the strophiolar tissue is sharply defined by the presence of a plate of brown-walled cells (*sp.* in the figures), having the same cytological characters as the epidermal cells of the integuments, which also become brown. In the young ovule the strophiolar plate is not to be seen, and has its origin at about the time the embryo-sac reaches its definitive condition, in scattered cells which marked the position of the plate, and which increase in number until the sheet of tissue is complete. When this takes place there are no fenestrations in the plate excepting a sometimes smaller one at the extremity of the chalazal branch of the vascular supply. It ultimately becomes continuous with the epidermis (*epi*, *fig. 9*) and thus the ovule exclusive of the strophiole comes to be completely surrounded by a sheath of cells with scanty plasmatic content, and deep brown walls.

In the third place the strophiole is characterized by the occurrence of a large number of excretory cells which become filled with raphides. When the seed is mature the strophiole carries a heavy load of calcium oxalate. As many as two hundred of the raphide cells have been counted. The remainder of the tissue is composed of cells which do not differ materially from the parenchyma of the integument. From the foregoing facts, it must be evident that the strophiole is the seat of special metabolic activity, during the growth of the embryo and endosperm, and that the strophiolar plate is of no special hindrance to the passage of proper food materials used by the embryo and endosperm.

ARCHESPORIUM AND EMBRYO-SAC

The archesporium is laid down in certain hypodermal cells of the nucellus of any early age. No tapetal cells are cut off, nor is

Mo. Bot. Garden
1903.

there any further growth of nucellar tissue above the archesporium. The conditions then, are precisely as in the *Galieae* in which the archesporium is surmounted only by a group of epidermal capping cells. In the form, here being described, the archesporium, however, contains only one functional megaspore mother-cell (*fig. 3*) surrounded by a number—about a half dozen—of cells of similar character, but reduced in size, and as their later history shows, possessing usually no ability to divide or to act as embryo-sac fundaments. No instance has been found in which two embryo-sacs have been present in a single ovule, a witness to the reduced ability of these cells as compared with their homologues in the *Galieae*.

The functional megaspore, however, grows apace and is easily to be recognized by its size and appearance. Its cytoplasm, especially at its outer end, is of a loose reticular structure which, in this form in particular, is very marked. Whether by division this cell divides to form megaspores or not, I can not at present say. In the figure shown (*fig. 3*) the synapsis stage there represented indicates that such divisions do occur since the first division of the embryo-sac nucleus takes place, in a closely related plant, at a later stage. To say that a direct development of the megaspore mother-cell into the embryo-sac takes place would, however, in the absence of positive evidence, be gratuitous. We therefore assume that such is not the case until a more favorable opportunity is presented for more careful investigation of the point.

The embryo-sac cell, when the integument is well grown, is ready to commence its development. It is to be noticed that in the case before us the subsidiary archesporial cells do not suffer disintegration during the enlargement of the functional embryo-sac cell. The latter when fully developed pushes forward, causing disintegration of the epidermal capping cells and enters the micropylar canal. Down this it moves, dividing meanwhile so as to give rise to the usual complement of embryo-sac cells. The remaining undivided megaspore mother-cells follow the moving embryo-sac cell, and arrange themselves lengthwise in the archesporial cavity and the adjacent portion of the micropylar canal in such a manner as to form a continuous strand of elongated cells

connecting the embryo-sac with the archesporial cavity (*figs. 4, 5, 12*). Near this point, as above pointed out, ends the chalazal branch of the vascular tissue of the strophiole. It must be further pointed out that the chalazal tissue cells are elongated between the archesporium and the vascular tissue, completing the path along which we may believe passes a supply of food materials from the parent plant to the developing embryo-sac. The persistence of the subsidiary archesporial cells with normal appearance until the endosperm is well developed sustains this view.

The embryo-sac in form is cylindric and presents, so far as the egg apparatus and endosperm nucleus are concerned, no point of special note. The fusion of the polar nuclei takes place quite near to the egg. The synergidae have well developed beaks which have been described for many forms and which I have referred to in another part of this paper.

THE ANTIPODAL CELLS

The feature of particular note is, however, to be found in the antipodal cells, which possess the power to divide, and form a tissue of considerable extent. The number of cells has been counted in several instances, and varies from four to ten. Frequently the several component cells are not separated by a cell wall, in which cases two or more nuclei may be found in a common mass of cytoplasm. The last antipodal is tapering in form, and overlaps the adjacent subsidary archesporial cells (*figs. 6, 12*), so that there lies, between the endosperm and the chalazal end of the vascular tissue, a continuous strand of transporting cells. There can be no doubt of the activity of the antipodal cells in this regard because of the following facts: The antipodal cells exhibit a degree of active growth, they are at all times richly supplied with starch, a substance which is abundant in the embryo-sac during its whole time of development, and they do not disintegrate, but, on the contrary, maintain their form and normal appearance for a long time after fertilization, while the endosperm is in its earlier most rapid growth. In appearance they are apparently similiar to the antipodal tissue described by Chamberlain (*l. c.*) in *Aster*. The coincident persistence of the archesporial cells is also noteworthy in this connection.

THE ENDOSPERM AND EMBRYO

The endosperm is at first parietal, and arises by free cell formation. At a somewhat early stage the central cavity becomes obliterated by the growth of the endosperm cells toward the center. Walls are then secreted and the tissue then presents in the main the same appearance as the endosperm in the *Galieae*. The peripheral cells, which are more densely filled with cytoplasm than the rest, appear to play the rôles of digestive and absorptive cells before which the integument breaks down as the endosperm grows. The integument breaks down with the usual appearances accompanying histolysis, although the cells of the micropyle resist the digestive action rather more than the rest of the tissue. The cells of the middle part of the endosperm, as the latter reaches a considerable size, present, on the other hand, a quite different character. They are larger and have contents which stain in such a way as to suggest that they also are suffering histolysis. A column of such cells extends from the embryo two-thirds of the longitudinal distance through the endosperm. As the embryo grows in length it comes to occupy this space, displacing the cells.

The embryo is of remarkably slow growth in the earlier stages of its development. The early growth takes place in the usual way, forming a suspensor of a few cells which are totally devoid of such outgrowths as have been described for the *Galieae*. The spherical form of the embryo proper is reached only slowly, and meanwhile the endosperm grows so as to reach nearly its final size. The later development of the embryo during which the cotyledons are formed is more rapid.

Diodia teres

(PLATE 12, FIGURES 13–17; PLATE 13, FIGURES 1–10)

THE NUCELLUS AND STROPHIOLE

With a trifling difference in the configuration of the nucelli, due apparently to the somewhat different mechanical relations in the ovary of this species, their behavior and that of the basal partition is the same as that in *Diodia Virginiana* (*pl. 12, fig. 13*). The insertion of the funicle, however, is not so far removed from the base of the ovary on account of the somewhat less rapid development

of the basal partition. Quite soon the strophiole appears on the concave side of the funicle (*pl. 12, fig. 14*), and the ridge gradually extends to the opposite side. With further development the micropyle becomes entirely closed by the strophiole, which agrees with its homologue in *Diodia Virginiana* in being possessed of special excretory cells containing raphides in large numbers, and in being the organ in which the vascular tissue of the ovule is distributed. The cells adjacent to the vascular tissue are small and are more densely filled with plasma, and increase in size in approaching the integument. The latter is not delimited from the strophiole by means of the brown cells which have been seen in *D. Virginiana*, and which are absent from the ovule entirely. The epidermal covering of the funicle and part of the strophiole is strikingly developed, and has been described more fully in another part of this paper. It is sufficient at present to point out the fact that they are deep columnar cells with thickened walls, and form a collar of tissue about the funicle, but having no relation to the nutrition of the ovule (*pl. 13, figs. 1, 7, 8*).

THE ARCHESPORIUM AND EMBRYO-SAC

The archesporium is in all respects similar to that of *Diodia Virginiana* (*pl. 12, fig. 15*). Only one functional megaspore mother-cell is present which divides to form four megaspores (*pl. 12, figs. 16, 17*). One of these, probably the basal, judging from appearances, such as are seen, for example, in *pl. 13, fig. 2*, is the embryo-sac cell. This grows rapidly, becomes loaded with starch, and commences its migration along the micropylar canal. As the nucleus passes forward, a vacuole is formed behind it. This vacuole is also present in *Diodia Virginiana* (*pl. 12, figs. 2, 4, and 5*).

The embryo-sac when mature is comparatively short, and only three antipodals are present, and we notice that, although one of these is much larger than the others, it is nevertheless much reduced in length as compared with that cell in the forms previously described. The persistence of these cells for a considerable period, together with their heavy starch contents indicate that they have a considerable amount of activity. The functional activity of the antipodal cells even in this form where, to all appearances,

they are of less importance than in the other plants studied, is indicated by the distribution of starch. In the unopened flower the embryo-sac is crowded with this food material, which is, however, absent from the rest of the ovule. As the flower opens, and at the time of fertilization, the starch disappears from the embryo-sac, first from the egg pole, so that in an old flower starch is entirely absent from the embryo-sac, but has commenced to accumulate at the chalazal end of the ovule. After fertilization, starch reappears in the antipodal cells, and then in the growing endosperm, so that when the latter has considerably increased (*pl. 13, fig. 6*), the former are very rich in starch content. In this connection it is to be noticed, also, that the subsidiary archesporial cells soon disappear, and take no further part in the development of the ovule. They are, in fact, quite lost to view when the embryo-sac contains but two cells. The endosperm nucleus lies appressed against the egg cell for some time before division. The endosperm is of the parietal type (*pl. 13, fig. 6*), and its behavior is similar to that of the same structure in *Diodia Virginiana*, as is also true of the integument.

The shape of the ovary is such that the chalazal end of the integument becomes folded over the strophiole, the whole producing the curious effect shown in *pl. 13, fig. 8*.

The embryo is slow in developing, and no haustorial outgrowths occur. The cells of the suspensor are at first short, and disc-shaped and later lengthen, bringing the embryo deeper in the endosperm (*pl. 13, fig. 9*). That the suspensor is not an organ of only mechanical significance may be inferred from the occurrence in it of a great deal of starch up to the time, at least, when the cotyledons are well started.

Richardsonia pilosa

(PLATE 13, FIGURES 11–18)

Material in cultivation at the New York Botanical Garden.

In the main, this plant agrees with the two species of *Diodia* as to the development of the ovule and archesporium, and of the embryo. The following important points of difference are to be noted.

The functional megaspore enlarges and develops into the

embryo-sac *in situ*. In lengthening, however, it grows forward, and destroys the nucellar cap, so that these cannot be seen after the embryo-sac nucleus divides. No migration, strictly speaking, takes place, so that the antipodal cells appear in the position previously occupied by the archesporium. The few reduced subsidiary megaspore mother-cells are soon absorbed and are lost to view.

By the usual number of divisions the eight embryo-sac nuclei are formed, and the egg apparatus, polar nuclei and antipodal cells arrange themselves in an oval cavity. The antipodals are constantly three in number, and are in this plant all nearly of a size (*figs. 14, 15*). For some time they possess a large, deeply staining nucleus, finely granular dense cytoplasm, and a vacuole at their free ends, and present the appearance of certain glandular cells, especially the stigmatic cells in some plants, and of synergidae. This fact is important, as it indicates a considerable degree of physiological activity on the part of the cells.

After fertilization the embryo develops slowly, forming first by transverse cell divisions a row of disc-formed cells. The suspensor remains always a short organ, with no special adaptations. The endosperm is at first parietal, but early fills the cavity by equal centripetal growth on all sides. At such time as this occurs, there appear at the apex of the embryo certain very much enlarged cells of endospermic origin, with nuclei considerably greater than those of the rest of the endosperm cells (*fig. 18*). Beyond their size, as just indicated, these cells do not present any peculiarities, and it is therefore difficult to any whether they possess any special physiological peculiarities, although this seems probable. They correspond in general to similarly placed cells in other types described, as having a centripetally developing endosperm, differing from them chiefly in size.

As the ovule enlarges after fertilization the limiting cells of the strophiole take on the brown coloration already noted in *Diodia Virginiana*. This change spreads to the cells surrounding the end of the vascular bundle. The peculiar function of these cells, if such they possess, remains problematical. It is certain, however, that they exert no unfavorable influence on the passage of food solutions, whether they have any positive value or not.

Houstonia

(PLATE 14)

The two species of the genus *Houstonia* which have been studied are *Houstonia coerulea* and *H. longifolia*, both of which have been found to be in essential agreement.

The style, which in *Houstonia* is single, is provided with two stigmas, and arises by the concrescence of two ridge-like fundaments which develop, as in the *Galieae*, laterally and opposite to each other, in the inside of the hollow torus (*s*, *figs. 1, 2 and 3*). During concrescence a transverse ridge (*figs. 1, 2 and 3*) is formed on the inside of the ovary roof, which, by downward growth, ultimately divides the originally single ovarial chamber into two locules. This ridge, which may be described as hanging from the roof of the ovary, unites along its free lower border with a parallel, low broader ridge developed in the floor of the ovary. This floor ridge (*b*, *figs. 2 and 3*), which must be regarded as representing two fused carpellary margins, is not evident until the two rounded fundaments of the placentae (*p*, *fig. 1*) have reached considerable size. These two structures have the same position and appearance as the fundaments of the ovules in the *Galieae*. In the latter forms, however, the two carpellary margins produce each a single ovule; in *Houstonia* a knob-shaped placenta is formed in each locule occupying the same topographic relations as the ovules in the *Galieae*.

Each placenta soon begins to show a number of protuberant growths, which may early be recognized as the primordia of ovules. The development of these is of peculiar interest.

Up till the time when the archesporium is to be distinguished the course of development of the ovule is like that in the *Galieae*. A single row of epidermal cells cap the archesporium, but these cells, which in the *Galieae* remain without further division, in *Houstonia* suffer periclinal divisions (*fig. 4*), as do also the adjacent epidermal cells. This process is continued until the archesporium comes to lie in the middle of the nucellar mass. *No integument at all is developed;* indeed, the regular periclinal cell divisions which characterize the early stage in the development of the integument in the *Galieae* are absent. The suppression of the integument accounts for the absence of the micropyle.

By growth in all directions the mass of the ovule is increased, and finally a bowl-shaped form is attained, with the concave surface turned toward the placenta. This change in form is accompanied by lengthening of the funicle, which has histologically the appearance of being a part of the placenta, but which in reality is derived by the regular division of the cells (*figs. 8 and 10, ff*), which constitute a very short stalk in the younger condition.

It is interesting to note in passing that the "*nucellus nudus*" of Schleiden, which that botanist incorrectly thought to be found in certain Rubiaceae, is realized in *Houstonia*.

It must be evident that generalizations made heretofore with reference to the occurrence of ovules without integument in degenerate plants must fall to the ground.

THE ARCHESPORIUM

The archesporium consists of a single large embryo-sac-mother-cell, surrounded by a single layer of usually about six small slender suppressed ones which show no tendency to divide. They, however, do not all degenerate, but some at least take on special cytological characters and take up their position in the cavity vacated by the functional embryo-sac-mother-cell in its development (*fig. 9*). In this figure one may see two such mother-cells which have the appearance of glandular cells, resembling in this regard the antipodal cells. From the same figure it is also evident that some of the mother-cells persist in this character for a considerable period, until, at least, the completion of the embryo-sac.

When the growth of the nucellar cap has proceeded until the archesporium comes to lie in the middle of the naked nucellus, the division of the megaspore mother-cell takes place. Four megaspores arise by two divisions, of which, contrary to the general rule among the Rubiaceae studied, the basal megaspore becomes the embryo-sac cell. In *fig. 6*, in which the arrow indicates the egg pole, are shown the four cells, of which the basal is the largest. Its sister cell is small, while the remaining pair of cells are evidently destined also to be of small dimensions.

The embryo-sac cell develops into the embryo-sac *in situ*. *fig. 7* shows the two-celled, and *fig. 8* the four-celled, condition. The definitive condition is shown in *fig. 9*. In its growth, the

embryo-sac destroys the surrounding nucellar tissue (*fig. 8*), but more rapidly in the direction of the egg pole, and leaving the tissue about the archesporium intact. The suppressed megaspores, which are represented on either side the functional one in *fig. 5*, meanwhile enlarge somewhat, and a vacuole appears in the cytoplasm. In this condition they may be seen, when the embryo-sac is mature, occupying the space previously mapped out as the archesporium, behind the antipodal cells, to which they are very similar in cytological characters, differing only in size. The antipodals are all of a size, densely filled with finely granular cytoplasm, and having a large vacuole in the end adjacent to the endosperm cells. Soon after fertilization both the megaspores, which persist up till this time, and the antipodal cells degenerate, and are seen as irregular, deeply staining masses.

Before the endosperm nucleus commences to divide it is seen lying against the young embryo. The growth of the endosperm is at first parietal, remaining so until two or three irregular layers of cells have been formed. As the result of the rapid increase in amount of vacuolation, however, the endosperm cells increase in size so as to obliterate the enclosed cavity, thus passing from the parietal condition to that found in the *Galieae*, but by a different mode of development (*figs. 10, 11, and 12*).

The development of the embryo is exceedingly slow. From the condition, in which it has a rather characteristic cylindrical form, it becomes multicellular by transverse divisions which occur slowly. No haustoria are produced, nor is there any point of particular interest in the later development.

When mature the seed consists of a seed coat of a few layers of very much compressed cells derived from the outer zone of the integument, enveloping the endosperm, which, in turn, surrounds the embryo.

The cells of the endosperm are rich in proteids with a few scattered starch grains. The cell walls are moderately thick, while those of the outer layer of cells are considerably thickened.

The separation of the seed from the placenta takes place at the plane of the insertion of the funicle in the seed, along the plane, therefore, in which the growth of the funicular cells by division takes place (*ff, fig. 10*).

SUMMARY AND CONCLUSIONS

The results presented in the foregoing pages have been derived from a study of 23 species representing 9 genera of the Rubiaceae.

In view of the great size and complexity of the family as at present understood, any conclusions based upon so small a proportion of the whole segregate may be applied with probable truth only to the genera represented by the species studied.

Of the lot, the *Galieae* have been the most thoroughly studied, and this group has been found to present a high degree of homogeneity, and the characters ascertained, with one exception to be noted below under 5 may be received as applying with only slight differences to all the plants of *Galieae*.

1. In all the plants considered, excepting in those of the genus *Houstonia*, two ovules are present. Their primordia arise in the floor of the ovary on either side the floral axis, and develop in such a manner as to produce approximately anatropous ovules provided with a single integument and with a greatly reduced nucellus which is not to be distinguished except at an early age, when it takes the form of a cap consisting of a single layer of cells crowning the archesporium. In the Compositae this layer forms a complete investment of an unicellular archesporium. It would appear, then, that the nucellus in these Rubiaceae is suppressed, a view however which is hardly in accord with the facts; for the apparent lack of a nucellus such as we find in the Compositae, may be regarded as a result of the thickening of the tissue of the primordium about the sides of the archesporium and the origin of the much thickened integument nearer the morphological apex of the primordium. According to this interpretation the nucellus is marked by the more abundant growth of its sides and its intimate relation to the integument.

This circumstance, if, as I hold, a fact, leads to a difficulty n delimiting the nucellus as ordinarily defined, a difficulty however no greater than that encountered in defining any organ when viewed in the light of modern morphological conceptions.

2. In the *Spermacoceae* there is, in addition to the integument, a second outgrowth, derived from the funiculus and here called a strophiole. This structure contains the vascular supply of the

ovules and possesses also in large numbers special excretory cells which become loaded with raphides.

Similar cells are found in some *Galieae* in the immediate vicinity of the embryo-sac for some time before and after fertilization, and the rôle which the contents play in the physiological economy of the ovule is by no means clear.

It may turn out that in both instances the calcium oxalate is of some positive value, and the term "excretory" as used above must be regarded as provisional.

3. In *Houstonia* each locule of the ovary is provided with a club-shaped placenta bearing each a number of ovules. The topographic relations of a placenta in *Houstonia* and an ovule in the *Galieae* are the same, and no differences have been observed in their origin.

The ovules in *Houstonia* are relatively very small and of a very simple type in that an *integument is wholly absent.* There is accordingly no micropyle and the archesporium becomes deeply buried in the nucellar tissue by the growth of the capping cells and of the tissue at the sides of the archesporium toward the apex of primordium. The archesporium further consists of but one functional embryo-sac-mother-cell.

Two questions arise concerning the above comparison; first, whether we may regard the ovule of the *Galieae* and the placenta with its ovules in *Houstonia* as morphological equivalents, and secondly, whether we have here a case of correlation.

To the first we can give no positive answer at present since a more careful study of the origin of the primordia is needed. If such should prove to be the case we should have a sort of branching or compound sporangium, a most interesting condition if true.

To the second question a more decided answer in the affirmative may be given in view of the fact that *where the placenta is formed the ovules are arrested* both as regards the integument and archesporium and in total relative size. That the archesporium is really suppressed appears from the fact that commonly about seven embryo-sac-mother-cells are laid down, all but one of which fail to reach the normal size.

It must be added that the belief that a simple naked ovule, that is, one without any integument, is correlated with the para-

sitic habit of growth, must now be thrown aside. This conclusion was suggested inferentially by Goebel* in connection with his observation on the ovules of *Crinum Asiaticum* of which he remarked: "Sie zeichnen sich durch eine, sonst nur bei Schmarotzerpflanzen bekannte Eigenthümlichkeit aus, sie sind nackt, es fehlen die sonst in Ein- oder Zweizahl vorhandenen Integumente."

4. The archesporium throughout contains a number (7–15) of megaspore mother-cells, the largest number occurring in the species of the *Galieae*. Each megaspore—barring the arrested ones at the side of the archesporium—divides twice to form four megaspores, which, except very infrequently, are not separated by walls. This condition is comparable to that described as occurring with some frequency in *Eichhornia* † and *Avena*.‡ All the megaspores are both morphologically and physiologically equivalent. It is, in consequence, impossible to pick out with the eye the functional megaspore, which appears usually to be the outer. As Koernicke § has recently emphasized, the embryo-sac cell in the great majority of Phanerogams is derived from the last megaspore, *i. e.*, the one at the chalazal pole, because usually the divisions which give rise to the megaspores are such as to give the largest proportion of plasma to that cell, a phenomenon which he holds to be connected with its more favorable position with reference to the source of nutriment. This view finds support in the facts here summarized in so far that the larger number of megaspores and undivided megaspore mother-cells form a nutritive tissue surrounding more or less completely the embryo-sac cell which arises near the longitudinal axis of the mass. That the functional megaspore, *i. e.*, the embryo-sac cell is found near the middle, and not in the sides of the archesporium is due, I hold, to the mechanical relation between the cells brought about by the growth of the ovule which is such as to produce no unequal pressure on the archesporium.

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* Biologische Schilderungen, 1 : p. 129.

† Smith, W. R. A Contribution to the Life History of the Pontederiaceae. Bot. Gaz. 15 : 324. 1898.

‡ Cannon, W. A. A morphological Study of the Flower and Embryo of the Wild Oat, *Avena fatua* L. Proc. Cal. Acad. Botany, III. 1 : 329. 1900.

§ Studien an Embryosack-Mutterzellen. Sitzungsber. der Niederrhein. Gesellsch. Natur- und Heilkunde zu Bonn. 1901.

That, in forms in which a pluricellular megasporangium is present, any or all of the megaspores have the ability to develop into embryo-sacs proves in a most convincing manner the morphological equality of the megaspores, and the regular division of each of the megaspore mother-cells into four megaspores must be regarded as a true tetrad division.

At this point it is of interest to compare this result with those reached by Murbeck * who has studied *Alchemilla*, a representative of the Rosaceae. The presence of a multicellular archesporium in this family was first made known by Strasburger. † According to Murbeck twelve to sixteen mother cells are present, of which the peripheral and usually one of the centrally placed, fail to divide, and moreover *never give rise to embryo-sacs.* The remainder, six to nine in number, divide at least once and of the daughter-cells some divide a second time, and the granddaughter-cells thus formed are regarded, properly, as megaspores. It may be noted in passing that here there is no reduced number of chromosomes, reproduction being parthenogenetic. Of the megaspores thus produced, two or more may develop into young embryo-sacs, and what is of immediate and special interest in this connection, *in the same sporogenous cell-row, two or more may commence their development into embryo-sacs.* It thus appears that *Alchemilla* and *Crucianella* are precisely comparable in this regard; while the evidence derived from the latter is still more final because of the actual division of all the megaspores derived from a simple mother-cell. Further cytological evidence that these are to be regarded as true tetrad divisions is presented on another page.

It is scarcely necessary to add that these facts accord an extraordinary strength to the view that the ovule is a sporangium.

In the *Spermacoceae* and *Oldenlandeae* the archesporium contains only one megaspore mother-cell of special size, from which the embryo-sac cell is derived. Several suppressed megaspores are present, surrounding the large megaspore mother-cell, which divides to form four megaspores, the basal of which forms the embryo-sac cell.

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* Parthenogenetische Embryobildung in der Gattung *Alchemilla*. Acta. Reg. Soc. Physiogr. Lund, 11 : No. 7. 1901. For a more extended examination of the relevant literature see this valuable paper.

† Die Angiospermen und Gymnospermen.

The appearance of a pluricellular archesporium may by no means be considered as primitive. It has been shown by several workers to occur in widely separated families,* and with certainty may be said to have no phylogenetic significance. On the contrary, the meaning is purely physiological, and such conditions have arisen in various forms in response to similar conditions. When present they offer favorable nutritive conditions for the chosen embryo-sac. This view comports at least with the case of *Alchemilla* as interpreted by Murbeck (*l. c.*). The archesporial tissue not directly concerned in the formation of the embryo-sac takes activity or passively a nutritive rôle. If their rôle is passive they gradually disintegrate, and are absorbed. This appears to be the case in the *Galieae*, *Diodia teres* and *Richardsonia*. In the *Diodia Virginica* they grow chiefly in length and arrange themselves as a transporting tissue. As such they connect the embryo-sac with the vascular supply of the ovule, and persist for a considerable period after fertilization. In *Houstonia* the subsidiary megaspore mother-cells appear to persist till about the time the embryo commences to develop, and undergo regular and constant changes in size and form. These changes indicate their physiological importance.

5. The embryo-sac cell behaves in two ways. In *Houstonia* and *Richardsonia* it develops *in situ*. In the *Galieae* and in *Diodia* it moves forward out of its original position, breaking through the nucellar capping cells, and passing along the micropylar canal. Its passage is intercellular, and its action is so far analogous to the movement of the pollen tube, as described by Murbeck and myself. As it moves it derives nutriment from the disintegrating cells adjacent to it. The disintegration is such as to suggest very strongly the secretion of an enzyme by the embyro-sac.

The embryo-sac presents some remarkable features as regards the antipodal cells. These, in the *Galieae*, with the possible exception of one species of *Asperula*, are three in number, one of which is very much elongated. The free end of this cell is plunged into the mass of disintegrating megaspores and absorbs the same. The physiological importance of all the antipodals is indicated by

*For full citation of the literature see the papers of Murbeck and Koernicke (*l. c.*).

their structure, persistence, food content, and the behavior of the surrounding tissues.

The genus *Crucianella* offers an exception to the general condition in the *Galieae*. Although antipodals are developed no evidence has been obtained that they take on any special cytological characters. Indeed their tenure of life is so short that the three antipodal cells have not been seen at the same time. Their function is probably, therefore, at best but passive, and is carried out by the mere giving up of their substance in death to the embryo-sac.

In *Diodia teres*, also, the number of antipodal cells is three, but present a difference in that the long one is relatively much shorter, a feature which is undoubtedly correlated with the smaller amount of archesporial tissue. They may, however, not be considered as physiologically inactive since they secrete a large amount of starch, and persist for a long time.

In *Diodia Virginiana* the number of antipodals is greater, the number varying, so far as observed, between four and ten. These are arranged in a long series, physiologically equivalent to the single long antipodal in the *Galieae*. The subsidiary megaspore mother-cells, as above pointed out, persist and form a continuation of the series. The antipodal cells retain their normal character for a considerable period covering the earlier period of endosperm development.

In *Richardsonia* and *Houstonia* the antipodal cells are equal in all regards, and appear to have less permanent value. They acquire the cytological characters of glandular cells such as are found in certain nectarial tissues and stigmas, and are probably active in some kind of secretion. Their relative small importance as judged by their size is connected with the behavior of the embryo-sac cell and the much suppressed archesporium.

That, in the words of Westermaier (*l. c.*, Part I), we have to do, in the antipodals, with "an anatomical-physiological apparatus, and not with a useless rudimentary structure which may be understood only from the viewpoint of comparative morphology," * is evident from the facts as here summarized:

* It is of great interest in this connection to recall the remark of Hofmeister made over fifty years ago: "Die am Chalaza-Ende entstandenen, durch ihre beträchtliche Grösse oft sehr ausgezeichneten Zellen scheinen keine andere Bestimmung zu haben, als die der Verarbeitung des Nahrungsstoffes für den werdenden Embryo." Die Entstehung des Embryo der Phanerogamen, p. 59. 1849.

(*a*) The form and cytological structure of these cells, and the changes of their food content.

(*b*) Their tendency in some plants to multiply and form a special tissue, of the nature of conductive or nutritive tissue.

(*c*) The behavior of the surrounding tissues notably the archesporial cells, which are absorbed.

We may not overlook the case of *Crucianella*, for which evidence is lacking that the antipodals play any but a very brief, passive rôle.

7. The embryo in the *Galieae* develops rapidly, especially in its early stage, and possesses a remarkable feature in the presence of a highly developed embryonic organ, the suspensor, the cells of which elongate laterally, and penetrate the surrounding endosperm in an intercellular manner without causing any disintegration of the endosperm. These outgrowths are termed haustoria. Analogous organs are known in a number of forms, and probably occur in many more in one form or another. The function of the suspensor in these forms is therefore not alone to bring the embryo into favorable position with relation to the food supply in a mechanical sense, but to act as a temporary embryonic root. This function is secondary, the former being regarded as primary, as for example in the case of *Selaginella* * in which it appears to have no further significance.

A strikingly analogous condition appears in certain species of *Tropaeolum*, according to Dickson,† in which the embryo produces "branches from the base of the suspensor" which "serve as organs of nutrition for the developing embryo—*foetal roots*, in fact." These organs penetrate into the pericarp.

Similar organs have been described by Treub ‡ in the Orchidaceae and by Hofmeister,§ followed by Guignard || in the Leguminosae.

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* Pfeffer, W. Die Entwickelung des Keimes der Gattung *Selaginella*. Hansteins bot. Abhandl. 1 : No. 4.

† On the embryogeny of *Tropaeolum peregrinum* (L.) and *T. speciosum* (Endl. and Paepp.). Trans. Roy. Soc. 17 : Part 2.

‡ Notes sur l'embryogenie de quelques Orchidees. Naturk. Verh. K. Akad. 19 : 1879.

§ Die Entstehung des Embryo der Phanerogamen, 1849.

|| Ann. sci. nat. VI. 12: 5. 1881.

It is, further, not improbable that the peculiar features of the suspensor in some Cruciferae, *Alisma* and many other plants are connected with similar physiological conditions.

The embryo in the *Spermacoceae* and in *Houstonia* develops very slowly, especially in its early stages. We have to note here the complete absence of the adaptive characters in the suspensor seen in the *Galieae;* the suspensor is of a very simple structure, and upon anatomical evidence alone we might attribute to it only the mechanical functions above indicated. In view of the distribution of food stuffs as already described, however, we must recognize the suspensor in these forms as a less special haustorial organ than in the *Galieae*, but still an organ of that nature.

It may further be noticed that the production of haustoria from the suspensor of the embryo, and, so far as known, from no other part, is probably an expression of polarity in the embryo.

8. The endosperm is of two kinds, according to its method of development, corresponding to two of Hegelmaier's * types.

In the *Galieae*, the endosperm during its whole course of development fills completely the endosperm cavity (Hegelmaier's first type). The peripheral cells on the basis of their cytological character and the behavior of the adjacent tissues may be regarded as concerned in the secretion of an enzyme, and in the absorption of the food derived by digestion of the reserve food substances in the integument.

In the *Spermacoceae* and *Houstonia* the endosperm is at first parietal, later coming to fill completely the enclosed cavity (Hegelmaier's second type). In addition to the peripheral cells, which appear much as in the *Galieae*, the centrally placed cells in *Diodia* and *Richardsonia* take on a more special character with a problematical significance.

THE TETRAD AND EMBRYO-SAC MITOSES

(PLATES 9 AND 11)

The following account of the mitoses of the archesporium and of the embryo-sac is based upon a study of *Asperula montana*, *Crucianella macrostachya* and *C. gilanica*. Of the two genera,

* Untersuchungen über die Morphologie des Dicotyledonen—Endosperms. Nova Acta Akad. Naturforscher., 49 : No. 1. 1885.

Crucianella offered by far the more favorable material both on account of the size of the cells and of the absence of unequal pressure due to peculiarities in the development such as occur in *Asperula*. The former has therefore received more exhaustive study, although in regard to certain points *Asperula* has given important results.

When the ovule is young the embryo-sac-mother-cells are to be distinguished only by their greater size. During the early development of the integument, however, or even earlier, their cytoplasm becomes denser. At about the same time a large number of fibers appear in the cytoplasm traversing the cells longitudinally but in somewhat irregular fashion. The fibers are more or less curved and taper off at their ends.

Inasmuch as the embryo-sac-mother-cells attain a strikingly great length before the first division takes place (*pl. 10, fig. 16*) and have their cytoplasm crowded with these fibers, they present a very unique appearance. In some cases the fibers traverse the cells from end to end, bending aside to avoid the nucleus. The condition here described apparently corresponds to that found by Mottier * and Dixon † in *Lilium*, and by Duggar ‡ in *Symplocarpus*, during the earlier development of the embryo-sac mother-cell, excepting in certain matters of detail and interpretation.

From the figures of Mottier and Duggar one would judge that the fibrillar structures result from a rearrangement of the cytoplasmic reticulum. That the large thick fibers found by me in the developing embryo-sac-mother-cell are formed in the same way seems to me not to be the case, for, in addition to their great thickness, I have not been able to see a pulling out or distortion of the adjacent cytoplasm. Reference will be made to a possible interpretation of these structures beyond.

Again, according to Mottier, the orientation of the fibers in *Lilium* is not constant in different cells of the same stage of growth. They may run in various directions or they may sometimes form a dense felt-like zone about the nucleus. Further, this cytoplasmic differentiation disappears before the commencement of mitosis, and

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* Ueber das Verhalten der Kerne bei der Entwickelung des Embryosackes und die Vorgänge bie der Befruchtung. Jahrb. wiss. Bot. 31 : 138. 1897.

† On the chromosomes of *Lilium longiflorum*. Proc. Irish Acad. III. 10 : 716.

‡ Studies in the development of the pollen grain in *Symplocarpus foetidus*, and *Peltandra undulata*. Bot. Gaz. 29 : 81. 1900.

by inference, has nothing to do with the formation of the mitotic figure. At the beginning of mitosis the cytoplasm shows again a uniform reticular structure, and the spindle of the first division takes its origin in radial or more or less irregularly placed fibers which penetrate the nuclear cavity, and form a multipolar spindle primordium.

With regard to the disappearance of the earlier formed thick fibers I am not able to establish a parallel in the Rubiaceae, for, in the embryo-sac-mother-cell in the plants studied they persist until the earlier stages of mitosis, at least through diakinesis. That they are in some way connected with the kinoplasmic figure during mitosis would appear from the circumstance that their ends on reaching the nucleus bend and run as figured in Mottier's *pl. 2, fig. 17*, of the paper referred to. This condition, as seen in *Crucianella macrostachya* is shown in *pl. 11, fig. 37*. From this and the adjoining figures it will be seen that the origin of the spindle is multipolar, and, with the possible exception of the peculiar behavior above described, corresponds to the descriptions of Osterhout * and others. The definitive spindle of the first division is strictly bipolar, and differs in the three plants in one or two details of form only.

In *Crucianella gilanica* the spindle is of very regular, broad, oval form, with sharply-pointed ends; in *C. macrostachya* it has a looser, more slender form, with attenuated ends, while in *Asperula* this character is still more accentuated. Such differences may in the main be regarded as inconstant and of no general importance, and may in many cases also be appearances referable to the plane in which the material happens to be cut. Insufficient observation of this point may very easily lead to false impressions, as Strasburger † has hinted, especially with reference to the character of the spindle poles.

Generally the ends of the spindle poles lie freely in the cytoplasm, inasmuch as the spindle axis usually lies parallel to the longitudinal axis of the cell, which, as already shown, is very long.

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* Ueber Entstehung der karyokinetischen Spindel bei *Equisetum*. Jahrb. wiss. Bot. 30 : 5. 1897.

† Ueber Reductionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich. Jena, 1900.

Occasionally, however, the spindle is found to lie obliquely, and one or both ends may become connected with the ectoplasm.*

In no case is a centrosome or centrosphere to be found. I have been able to follow finely-pointed spindles to their extreme end without finding any trace of the body which can without doubt be regarded as such. In a recent paper by Meves and von Korff on the Myriopoda,† the "centralkörper" are described as lying very near to the periphery of the cell, and are apparently not directly in connection with the spindle. The latter, however, is not pointed, while its fibers nevertheless are directed toward the kinetic centers.

Thus is formed a condition in which the centrosomes are considerably removed but still in control of the spindle ends.

In spite of the obvious difference in the character of the spindle itself, I was led to examine carefully every part of the cytoplasm lying in or near the spindle axis, but with a negative result.

The multipolar origin of the spindle in certain plant cells has been for some time regarded by the majority of botanists as speaking against the existence of the centrosome in plants in which the spindle has such an origin. Although a contrary view has been held by Guignard‡ it would still appear that the former position is more consonant with the facts as they are at present understood.

For we would expect in such cases either as many centrosomes as spindle ends, or two whose influence would be exerted over the whole kinoplasmic figure (archoplasm). The former is shown to be the case in the development of the curious worm-shaped spermatozoon in *Paludina*, an account of which has lately appeared.§ According to Meves the centrosphere of the primary spermatocyte contains a number of central granules which act as a unit. In the second mitosis the granules (Centralkörperkörner) separate and take a position in the periphery of the cell, and from these as centers are formed a number of half or partial spindles. The result is a picture which resembles in its later development to some

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* Ectoplasm is here used to signify the "Hautschicht" of the Germans.

† Meves, Fr, and von Korff, K. "Zur Kenntniss der Zelltheilung bei Myriopoda. Archiv mik. Anat. und Entwickelungsgeschichte, 57: 481–486. *pl. 21.* 1901.

‡ Centrosomes in Plants. Bot. Gaz. 15: 158. 1898.

§ Meves, Fr. Ueber die sog. wurmförmigen Samenfäden von *Paludina* und über ihre Entwickelung. Mitth. Verein Schles.-Holst. Aerzte. 10: No. 1. 1901.

degree the multipolar spindle primordium in plants, to which the author unhesitatingly compares it, with the inference that the plant cytologists have still overlooked the structures in question. But the comparison is without warrant, for an examination of the facts shows that we are dealing with two different modes of action.

The origin of the spindle is in the two cases quite different. In *Paludina* it is in the periphery of the cell, while in the higher plants it is at the nucleus. The development of the spindle is in the former therefore centripetal; in the latter centrifugal. We fail therefore to see any warrant for the conclusion that there is any parallel between the phenomena described by Meves, and those seen in the formation of the plant spindle of multipolar origin.

The parallel fails also in the following regard. In *Paludina* the spindle fibers develop from the centers. In plants, even admitting the existence of centers, the fibers are developed to a very considerable degree before the centrosomes, or centrosome-like bodies, when such are recognized, appear.

For the present, therefore, we must adhere to the view that the centrosome is absent from the higher plants.

We do not attempt to meet in detail the statements in certain recent papers to the contrary. * It is quite impossible to do this except by working with the same materials and methods, and this has not been done by me.

Nor, as Strasburger (*l. c.*) has shown, is positive evidence against the occurrence of the centrosome in higher plants wanting. Repeated studies on pollen development have been carried out, showing that the ends of the spindle find an insertion in the ectoplasm and the results of my own observations tend strongly to confirm the view that the ectoplasm serves in many instances as a place of connection in those cells in which the spindle is long enough to bridge the distance from side to side. One instance (*Asperula*) was seen in which a pluripolar diarch spindle had its four apices (appearing in a single section) were all inserted in the ectoplasm (*pl. 9, fig. 16*).

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* Bernard, Ch. Recherches sur les sphere attractions chez *Lilium candidum*, *Helosis guyanensis*, etc. Jour. de Bot. 14: 118, 177, 206. 1900.

Yamanuochi, S. Einige Beobachtungen uber die Centrosomen in den Pollenmutterzellen von *Lilium longiflorum*. Bot. Centralbl. Beihefte, 10: 301. 1901.

Schaffner, J. H. A Contribution to the Life History and cytology of *Erythronium*. Bot. Gaz. 31: 369. June, 1901.

When, however, the true apex of the spindle does not insert itself immediately in the ectoplasm, one may usually discover a fine strand of kinoplasm extending from the apex of the spindle to the ectoplasm (*pl. 9, fig. 10*).

In further support of the view that the spindle often stands in some intimate relation with the ectoplasm I may cite the instance from which fig. 4 of Pl. 9 was taken. The axis is here oblique to the optical plane, and one pole is seen to end distinctly at a point from which extend fibers in a somewhat radial fashion. Careful examination revealed the fact that the radially placed fibers lay in the peripheral layer of the cytoplasm, that is in the ectoplasm, and the latter therefore appears to bear a distinct mechanical relation to the spindle.

In many cases in which the dimensions of the cell are much greater relatively to the size of the spindle such attachments have not been found. This lack is especially obvious in the large embryo-sac-mother-cell of *Crucianella*, in which, however, as in the pollen spindles, extra-nuclear "supporting fibers" may be observed to run out from the spindle apices to the ectoplasm in the direction of the equator (*pl. 11, figs. 16, 17*). Similar conditions prevail in the pollen mother-cell in *Crucianella macrostachya* (*pl. 11, fig. 35*), where but a single row of sporogenous cells occurs in each locule of the anther. The cells are relatively large and the mitotic figure in both the first and second divisions lies freely in the cytoplasm. In this instance neither the insertion of the spindle poles, nor of the extra-nuclear supporting fibers in the ectoplasm could be seen with certainty. Indeed the orientation of the spindle fibers during the second division is often such as to preclude the possibility of the insertion of one of the poles at least, as will be seen in *pl. 11, fig. 35*, which shows that the axes of the two spindles lay in the same plane at right angles to each other; unless, indeed, we may expect to find the pole of one spindle passing through the body of the other.

In the developing pollen in many plants a distinct zone of very granular cytoplasm has been described by Juel,* Lawson, †

* Die Kerntheilungen in den Pollenmutterzellen von *Hemerocallis fulva* und die bei denselben auftretenden Unregemässigkeiten. Jahrb. wiss. Bot. 30 : 204. 1897.

† Some observations on the Development of the Karyokinetic Spindle in the Pollen mother-cells of *Cobaea scandens*. Proc. Cal. Acad. Botany, III. 1 : 169. 1899.

Byxbee, * Williams, † and others, and it has been suggested by Strasburger that, in those spindles of which the ends do not reach the ectoplasm, or in the absence of other mechanical support, the pericaryoplasm (of Lawson) may serve that purpose.

I have observed this zone in the plants under consideration, but in instances the poles of the mitotic figure reach the ectoplasm (*pl. 11, fig. 2*). In this event the zone thins out as it approaches the spindle pole. In the pollen mother-cell of *Crucianella macrostachya* this zone has not been observed and the spindle appears to lie quite freely and without any special mechanical relations.

The amount of variation in the observations of the topographic relations of the spindle ends leads to the suspicion that we are yet unable to form a satisfactory explanation of them. The most far reaching interpretation among the botanists is that of the Strasburger school, and is based upon the mechanical conception that the spindle is made up of contractile fibers (Zug-fasern) which, in order to pull apart the chromosomes, must have a more or less rigid mechanical support. This has been found in the ectoplasm, in the pericaryoplasm, in the vacuoles,‡ or the spindle ends may be buttressed by extra-nuclear supporting fibers.

That such mechanical relations as described by Strasburger and others really exist we may not doubt. All the appearances lead irresistibly to that conclusion. But I am by no means convinced that we may infer from these appearances anything as to the intimate workings of the mechanism, and the contractile fiber explanation may have to undergo perhaps a considerable degree of modification before it may be accepted as satisfactory.

Reference has already been made in another part of this paper to the absence of cell walls between the megaspores. It will be seen from *pl. 11, fig. 20*, and *pl. 9, fig. 23*, that the connecting fibers are formed, and the thickenings indicative of the cell plate are present. In *Crucianella* the presence of these fibers determines the appearance of the cytoplasm for some time (*pl. 10, fig. 9*). At the end of the third division in the same plant the nuclei are

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* The Development of the karyokinetic Spindle in the Pollen Mother-cells of *Lavatera*. Proc. Cal. Acad. Botany, III. 2 : 63. 1900.

† The Origin of the karyokinetic Spindle in *Passiflora coerulea* Linn. Proc. Cal. Acad. Botany, III. 1 : 189. 1899.

‡ Strasburger, l. c.

arranged sometimes in two rows, and the connecting fibers run from one nucleus to the other in such a manner as to recall Mottier's* figure of the four nuclei in the upper end of the embryo-sac in *Helleborus foetidus*, and the appearances which one usually meets with during the formation of cell walls in endosperms (*pl. 10, fig. 18*).

The prophase of the first division in both pollen and embryo-sac-mother-cells is characterized by the stage known as diakinesis (of Häcker), in which the chromosome pairs become condensed into short, thick rods which lie parallel or become partially twisted about or crossed upon each other. The members of each pair may fuse at one or both ends, and as the result of more or less separation in the middle, may take also the form of rings either completely closed, or open at one point. Between these more definite forms the chromosome pairs may assume all sorts of intermediate conditions (*pl. 11, figs. 1, 14, 32, 37, 38*).

In some instances the members of the chromosome pairs may be separated from each other for a considerable distance, and the linin strands which unite their ends, may under such conditions be seen with the greatest clearness (*pl. 9, fig. 21, a*). In *Galtonia candicans*, according to Schniewind-Thies† the same behavior has been noted.

The formation of the rings takes place much more completely and earlier in *Asperula* in which genus also the condensation of the large loose rings into small thick ones may be followed with ease (*pl. 9, figs. 1 and 2*). The appearances so presented recall the figures of vom Rath‡ of the chromosome rings in *Gryllotalpa* excepting, however, that no phenomena of tetrad formation (in the sense of the zoologists) may be observed.

During the prophase of this division the number of chromosomes may in these forms quite easily be determined and with a high degree of certainty has been found to be *one-half the usual number in the sporophyte*. The reduced number, which has been determined by counts, not only in the prophases, but in the ana-

* Ueber das Verhalten der Kerne, etc. Jahrb. wiss. Bot. 1897.

† Die Reduction, der Chromosome Zahl und die ihr folgenden Kerntheilungen in den Embryosacmutterzellen der Angiospermen. Jena, 1901.

‡ Zur Kenntniss der Spermatogenese von *Gryllotalpa vulgaris*. Arch. Mic. Anat. 1892.

phase of the first and second pollen division and in the first, second, third and fourth embryo-sac divisions, is in *Crucianella* the hitherto unobserved and surprising number ten, in *Asperula*, twelve.

These numbers, however, are believed, on the basis of a large number of counts at different times, to be inconstant. In *Crucianella* eight to eleven have been counted, and in *Asperula* a similar amount of variation has been observed. Allowing for error in counting, which, in these plants is not great, both on account of the smallness of the cells (with the consequent frequency with which the whole nucleus appears in a single thick section) and of the number of chromosomes, I believe it must be maintained that *variation in this regard occurs*, and that this is a fact which has heretofore received insufficient recognition.

The condensation of the chromosome pairs proceeds all through the prophase until the equatorial plate has been completed, when they take the form of a double Vs, one of which lies above and one below the equatorial plane, with their angles directed toward the axis of the spindle. This arrangement is difficult to recognize in metaphase on account of the extreme condensation of the chromosome, but in very early anaphase may be seen with sufficient distinctness.

My observations and conclusions appear to coincide with those of Duggar* for *Peltandra*. That author's interpretation of his figures of the corresponding division in *Symplocarpus* as indicating a qualitative division, however, would in my judgment seem not to be justified. For the chromosomes are here, as represented in *pl. 1, figs. 7 and 7a* of Duggar's paper, of double V-form with the angle directed toward the center of the spindle. These separate first at the angle, and the degree of similarity in the two plants is so great as to render them susceptible of but a single interpretation, which is opposed to the view that there is a qualitative division, unless it can clearly be shown that the separation of the limbs of the V is a separation, not at their ends, but across the middle of the chromosomes.

Attention should further be directed to the above mentioned observation, namely, that in diakinesis and the later prophase the

* Studies in the development of the pollen grain in *Symplocarpus foetidus* and *Peltandra undulata*. Bot. Gaz. 19: 81. 1900.

ends of the chromosome pairs do not invariably fuse even when they take the form of rings. With the approach of metaphase the two members if separated become laid together. The fixation of the fibers then takes place somewhere between and not at the ends of the chromosomes, so that the possibility of the occurrence of a transverse splitting of the chromosomes at this time, as suggested most recently by Schaffner * for *Erythronium*, seems to me to be excluded.

If the ends of the chromosome pairs always fused completely it would probably be impossible to tell whether the breaks during anaphase occurred at the points of fusion or somewhere between these. When, however, such a fusion does not take place the simpler and more obvious interpretation does not involve the idea of a reducing division.

The separation of the daughter chromosomes takes place with extreme regularity but curiously enough with quite constant differences in the two genera. In *Asperula* the ends of the daughter Vs separate simultaneously and the chromosomes take with little variation the form of a conventional heart. This is to be explained by the shortness of those bodies and the point at which the fibers are inserted (*pl. 9, figs. 3, 22*).

In *Crucianella*, on the contrary, the ends of the V-formed segments scarcely ever separate at the same time. The ones which remain united the longer are attached to each other with so great a degree of firmness that it is only after the greater mass of each daughter chromosome reaches about half the distance to the spindle poles that they finally separate (*pl. 11, figs. 2, 3, 16, 17, 18, 39*). The forms taken by the separating chromosomes under these conditions have received special study, and all of them suggest very definitely the behavior of plastic masses under tension. Examination of the detailed representations on *pl. 11* (*fig. 3, a–f; fig. 18, a–g*) will reveal this point quite clearly. Especially instructive are such forms as shown in *fig. 18, a*, as they bear on the question of transverse division. Were the chromosome here breaking transversely and not undergoing separation at united ends, it is difficult to see why such a bulge at the point of separa-

* A Contribution to the Life History and Cytology of *Erythronium*. Bot. Gaz. 31 : 369–387. 1901.

tion should occur. On the other hand, it is quite easy to understand such an appearance on the supposition that a plane of adhesion exists at that point.

Whether the unequal separation of the daughter chromosomes is due to the failure of the mantle fibers to exert an equally distributed pull upon them, or to the fact that the fusion of the chromosome ends is not equal cannot be determined. It is a further point worthy of note that the two free ends of an incompletely separated chromosome pair are always directed away from the spindle axis, as if the mantle fibers were stretched along a resistant surface. After the separation of the daughter chromosomes is complete, a readjustment of tensions takes place so that the heart-shaped form spoken of in connection with *Asperula* is approached (*pl. 11, figs. 4, 16, 17, 18, g*).

During late anaphase a second longitudinal splitting of the chromosomes is seen with a fair degree of clearness (*pl. 9, figs. 6–9*). On account of the shortness of the resulting chromosomes, however, it is not easy to determine the forms which they take. For the most part they appear as pairs of straight rods, although some evidence that they are slightly bent into a V is seen. It is not at all improbable that a considerable amount of readjustment in the relations of mantle fibers and chromosomes takes place and this would explain the change in the shape of the chromosomes.

The appearances here described have been constantly seen in a large number of cells and there is very little doubt that the first division in *Asperula* is heterotypic in the sense adopted by Strasburger (*l. c.*, 99).

The corresponding longitudinal splitting of the chromosomes in *Crucianella* does not take place so early as in *Asperula*, and may be seen with clearness only in the telophase. *Pl. 11, fig. 5*, represents such a stage. It appears not possible to resolve the chromosome pairs thus formed during the whole of the period extending from this time till the metaphase of the second division; for as shown in *pl. 11, Fig. 6*, the pairing is indistinguishable, although a completely resting condition, as described for *Larix* by Juel,* in

* Juel, H. O. Beiträge zur Kenntniss der Tetradtheilung. Jahrb. wiss. Bot. 35: 626. 1900.

Pellia, by Davis,* in *Asclepias*,† by Strasburger, and in certain Liliaceae by Schniewind-Thies ‡ has not been observed to occur.

From the facts above presented it will be seen that in *Crucianella*, as in *Asperula*, the first splitting of the chromosomes is longitudinal. In early anaphase the daughter rods which open out from a double V-form into rings, separate finally at the ends of the limbs of the V, the angles being directed toward the pole. Later a second longitudinal splitting appears, the formation of granddaughter rods being completed before the close of the first mitosis. This division is, therefore, heterotypic and corresponds in essential details to the heterotypical mitosis in the spermatocytes of the salamander as described by Flemming.

The final evidence for this conclusion in regard to the embryo-sac-mother-cell has not been obtained without any doubt, but I believe that we may conclude from the otherwise complete correspondence in the two cases that the first embryo-sac-mother-cell division is also heterotypic.

The resting stage occurring between the first and second divisions is characterized by its short duration, and by the varying degree of diffusion of the chromatin. This variation is considerable not only among related species, but is evident in comparing the pollen and embryo-sac tetrad divisions in the same plant, as Strasburger § has observed to be the case in *Lilium*. It is notable that in the Rubiaceae also the daughter nuclei of the pollen mother-cell enter the resting stage less completely than in the corresponding nuclei in the ovule. If this prove to be generally true we may attribute the behavior to the closer analogy between the tetrad divisions in pollen and those of cryptogams, and the greater irregularity in the tetrad divisions in the ovule to the changed conditions which have been brought about in that organ.

Although the variation in the character of the resting stage is

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* Davis, B. M. Nuclear Studies in *Pellia*. Ann. Bot. **15**: 147. 1901.

† Einige Bemerkungen zu der Pollenbildung bei *Asclepias*. Ber. deutsch. bot. Gesellsch. **19**: 450. 1901.

‡ Schniewind-Thies, T. Die Reduction der Chromosomezahl und die ihr folgenden Kerntheilungen in den Embryosacmutterzellen der Angiospermen. Jena, 1901.

§ Ueber Cytoplasmastructuren, Kern und Zelltheilung. Jahrb. wiss. Bot. **10**: 252. 1897.

real and considerable, all the cases studied are alike in the absence of a nucleolus, in the indistinctness of the nuclear membrane, which appears as if surrounded by an ill-defined zone of fine kinoplasmic fibers running in various directions, and in the loss, at least so far as direct observation can take us, of the individuality of the granddaughter chromosomes, even when, as in *Crucianella*, the second splitting takes place in early telophase.

The occurrence of a more or less complete resting stage at the end of the first division is a phenomenon of wide occurrence among the phanerogams. Authors are not agreed, however, as to the degree into which the daughter nuclei enter at this stage. More recently Schniewind-Thies has found a considerable amount of variations in a number of forms studied comparatively by her. It appears, therefore, that we may explain this disagreement on the ground of the occurrence of a considerable degree of variation in this regard.

It does not appear to be necessary to be concerned about the loss of individuality by the chromosomes under the circumstances at least so far as the question of reduction is involved. A second longitudinal splitting being demonstrated to occur before this takes place, we are compelled to believe in the absence of positive evidence to the contrary that the rehabilitation of the chromosomes is complete. The Weissmannian interpretation once shut out, it remains for us to explain the variable degree into which the granddaughter nuclei enter the resting condition apart from it. I cannot agree with Davis, nor do I find for him any justification in the literature of the subject, either on the zoölogical or botanical side of the question, when he remarks "This conception * is intimately concerned with, and necessarily a part of, the recent studies of Guignard and Strasburger on the double longitudinal splitting of the chromosomes." (*l. c.*, p. 159.)

Indeed Strasburger himself has recently shown that in *Asclepias* (*l. c.*) a complete resting stage, with a nucleolus, is formed between the first and second pollen divisions, without making any remark further than to call attention to the fact.

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* That the chromosomes maintain their individuality in the daughter nuclei after the first mitosis of the pollen mother-cell.

THE SECOND DIVISION

During the prophase of the second division in the embryo-sac-mother-cell delicate radiating fibers in great numbers are found surrounding the nucleus and extending from the same in all directions (*pl. 11, fig. 42*). In certain cells where the walls come into contact with the nucleus these fibers then appear in sections as if there were two distinct systems arising from the opposite sides of the nucleus. They thus simulate asters. In point of fact, however, they form a zone about the nucleus lying parallel to the compressing cell walls (*pl. 11, fig. 15*).

Such radiations have been figured and described by many authors, but accounts are, in spite of a multitude of detail, still in such a condition as not to warrant us in believing that we as yet understand fully the relation between the nucleus and these radiations.

Their resemblance, however to certain appearances is parthenogenetically developing eggs of *Toxopneustes* after treatment with magnesium chloride has led Wilson * to make the following suggestion which I give in his own words.

"The formation of a perinuclear zone in the magnesium eggs strongly suggests the accumulation of 'kinoplasm' about the nucleus in the early prophase of cell division in the higher plants, as described by Osterhout and Mottier, and a number of later observers, though the later phenomena are in many respects very different."

According to Wilson's direct observations on the living Mg-eggs, a primary radiation appears surrounding the nucleus and extending far out to the periphery of the cell. This radiation is interpreted as a flow of hyaloplasm which, in part at least, gives rise to the appearances making up the "aster." The gradual disappearance of these radiations is accompanied by the formation of a clear perinuclear zone, followed by "enlargement of the nucleus and the disappearance of the nuclear membrane accompanied by a reduction of the rays almost to the vanishing point." (*l. c.*)

The comparison is strengthened by the facts described earlier

* Experimental Studies in Cytology, I. A cytological Study of artificial Parthenogenesis in Sea-Urchin Eggs. Archiv Entwickelungsmechanik der Organismen. **12**: 529. 1901.

in this paper in connection with the development of the embryo-sac-mother-cell. The numbers of fibers then seen and their apparent behavior with respect to the nucleus, as already indicated, in many ways harmonize with Wilson's suggestion. If further study should show this harmony to be complete, we would then have strong grounds, in the absence of direct observation of the living material, for the belief that these fibers are currents of viscid material (kinoplasm, hyaloplasm) the view first taken by Auerbach, and later adopted by Fol, Strasburger † and others in modified form.

It would appear that a further and more particular study of the behavior of the kinoplasm during the prophase in plants from the suggestive point of view just indicated would have a distinct bearing upon the vexing problem of the nature of the fibers.

In the prophase of the second division the granddaughter chromosomes reappear in *Crucianella* as pairs of rods lying parallel to or partly twisted about each other and lying on the nuclear walls (*pl. 11, fig. 21*). In *Asperula* the chromosomes are so short that they appear as pairs of spheres (*pl. 9, fig. 16*), though in metaphase they frequently appear as pairs of short rods, one lying above and one below the equatorial plate. The mantle fibers are usually attached to the chromosomes at their ends, one chromosome lying above and one below the equatorial plane (*pl. 11, figs. 8, 22 and 35*). Occasionally, however, they may be attached at some point along their length. During anaphase, therefore, the chromosomes have the form of short rods with their longitudinal axes directed toward the spindle poles (*pl. 11, fig. 24*), or less frequently of bent rods with the loop similarly directed (*pl. 11, fig. 25*). There are some minor differences between the form of the chromosomes in the second pollen- and embryo-sac-mother-cell divisions. In the latter the rods are longer and more slender (*pl. 11, fig. 24*); in the former shorter and thicker (*pl. 11, fig. 9*). On account of this fact and the further one that in *Crucianella gilanica* the spindles are very small in the pollen cells, the character of the structures is rather difficult to determine. In *Crucianella macrostachya*, in the anther locules of which but one row of pollen mother-cells is present, the opportunities

† Zellbildung und Zelltheilung. 3 Aufl., 367.

for observations are better, and here the chromosomes are seen distinctly as paired rods, with the fibers for the most part attached to their end (*pl. 11, figs. 35 and 36*).

The contrast offered between the appearances seen during the first and second divisions are well seen in *pl. 11, figs. 16 or 17, and 24*, all of which have been taken from the same ovule. The spindles shown in *figs. 16 and 24, pl. 11*, may indeed be seen in the same field in adjacent megaspore mother-cells.

According to the interpretation here given of the behavior of the chromosomes the application of the facts to the Weissmannian view of reduction is made impossible.

That, however, a real reduction in the quantity of chromatin occurs as well as a reduction in the number of chromosomes has been held by Guignard, according to whom the second division which follows on the first without pause reduces the nuclein which the grand-daughter nuclei receive by a half, compared with the amount which the ordinary nuclei of the sporophyte have. Such reduction, however, results in equivalent cells as regards their hereditary qualities.

A reduction in this sense certainly occurs in the plants under consideration, if we may argue from the relative sizes of the nuclei. The figures which present the facts, namely, *pl. 11, figs. 6 and 13*, are drawn to the same scale and show that the nuclei at the end of the second division are much smaller than those at the end of the first. True, the cells themselves are also smaller and this indicates that the cause of such quantitative reduction is to be found in the rapidity with which the divisions follow each other, thus preventing an intervening period of growth.

THE THIRD DIVISION

The resting stage at the close of the second division is complete, a nucleolus is formed, and a considerable pause intervenes before the third division takes place.

It has been already pointed out on another page that in the genus *Crucianella* the megaspores are unique in that they are all functional in so far that they frequently undergo the first embryo-sac division. All the divisions which take place in this way are alike in character, that is they are typical mitoses. The further

divisions of the supernumerary megaspores have not been observed in any other genus, and in all probability do not take place. It would appear from comparative study that the large size of the megaspores and the absence of compression by the surrounding tissues offer favorable conditions for the access of food materials and the equally rapid development of all the daughter and granddaughter cells, thus giving them an equal start as embryo-sac cells. The embryo-sac cell which finds itself in the most favorable position as regards the micropylar canal then commences a more rapid development, which takes place at the expense of the remaining megaspores. In the prophases of the third and succeeding divisions the chromosomes are about twice as long as those in the second division, the fibers are usually attached at about their middle point, where they are bent at a slight angle; the angle is directed toward the center of the spindle (*pl. 11, figs. 27, 30*). This division conforms, therefore, to the ordinary vegetative or typical division, differing only in the possession of the reduced number of chromosomes, which have been determined to be in *Crucianella* ten. It is not necessary for our present purpose to describe the remaining divisions in detail, more than to say that they fully correspond in character to the third.

The embryo-sac divisions in *Asperula* were not studied.

SUMMARY AND CONCLUSIONS

1. During the growth of the embryo-sac-mother-cell before the first division a large number of coarse fibers are present in the cytoplasm which persist through diakinesis of the first division. These are, in part at least, continuous with the circumnuclear complex of fibers preceding the formation of the spindle, and do not result from a rearrangement of the cytoplasmic reticulum. Further it is suggested that they be interpreted as currents of kinoplasm (hyaloplasm) corresponding to the radiations described by Wilson as occurring in the sea-urchin egg.

2. The spindle in both pollen- and embryo-sac-mother-cell is of multipolar origin. The nucleus is at first surrounded by a feltwork of curved tangentially placed fibers which gradually segregate into conical groups. By fusion of these groups a bipolar spindle is formed.

3. Centrosomes or centrosome-like bodies are not present. The poles of the spindle are finely tapering points with various topographic relations described in the body of the text. The suggestion made by Meves, prompted by his observations on *Paludina*, is not pertinent.

Under certain conditions, as for example in the pollen-mother-cell, where the proportions of the cell are not too great relatively to the size of the nucleus, the spindle ends find an insertion in the ectoplasm. At the point of insertion a small thickening may frequently be seen, and from this point run out radially placed, branching fibers in the ectoplasm. It is suggested that these are currents in the ectoplasm and may represent kinoplasm passing from the spindle into the ectoplasm during anaphase.

4. At the end of the first and second embryo-sac-mother-cell divisions, and at the end of the third division also when such occurs, as is generally the case in *Crucianella*, a system of connecting fibers arises without, however, the formation of cell walls. The megaspores and their derivatives in *Crucianella* remain in a syncytial condition.

5. A reduced number of chromosomes appears in the prophase of the first division of the pollen- and embryo-sac-mother-cells; in *Crucianella* this number is ten; in *Asperula* twelve. These numbers are maintained throughout the subsequent divisions.

The prophase of this division is characterized by the stage known as diakinesis. The metaphase offers on the whole the same characters as have been described by a number of authors for various plants and differs from them only in points of detail referable to the proportions of the chromosomes. The frequently difficult observation of the so-called ring form of the chromosome pairs in metaphase is a case in point. When the chromosomes are relatively more slender, as in the Liliaceae, the appearances are relatively more distinct. In many other plants they are less so on account of the extreme shortness and thickness of the chromosomes.

The second splitting of the chromosomes is longitudinal, and takes place in *Asperula* during the late anaphase of the first division. In *Crucianella* a similar splitting of the chromosomes takes place in the telophase of the first division and is here also longitudinal.

The first division in these plants is therefore heterotypic, in the sense of Flemming and Strasburger, and the second is homotypic, and results merely in the separation of the granddaughter rods already formed. The individuality of these bodies is, however, lost, so far as can be seen, in the resting nucleus preparatory to the second mitosis by fusion of the rods and by more or less complete diffusion of the chromatin.

It is concluded from this evidence that the divisions described are true tetrad divisions, and the four resultant cells as spores. It has on several occasions been urged that the position of these cells in the nucellus of angiosperms speaks against this interpretation, but it is not surprising that such an arrangement should occur in the ovule when we remember that the position of the spindle and through the spindle often of the cell wall is determined by growth, and that the growth of the sporogenous tissues is conditioned by a very different sort of environment from that found in the sporangium of the pteridophyta.

But the evidence is fast accumulating to show that it really frequently happens that the divisions are not such as to produce a single row of cells. This evidence has recently been collated by Max Koernicke * to which may be added the cases recorded by G. M. Holferty † as occurring in *Potamogeton*.

Adding to the above the facts derived from a cytological study of the divisions themselves, I fail to see the cogency of the statement made by Atkinson ‡ that the embryo-sac of the angiosperms "arises directly from the nucellar (sporangial) tissues or from the archesporium, without the intervention of spores." That the spores in many forms are losing or have already lost their physiological identity as spores is indeed true, but it is certainly a most remarkable fact that cannot be brushed aside that in the most extreme cases *Lilium* for example, the process of giving rise to these, cells is identical in all essential respects, to the process in all other plants, both pteridophyta and spermatophyta, in which this point has received careful study.

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* Studien an Embryo sac Mutterzellen. (*l. c.*)

† Ovule and Embryo of *Potamogeton natans*. Bot. Gaz. 13 : 339. 1901.

‡ On the Homologies and probable Origin of the Embryo-sac. Science, II. 13 : 530. 1901.

On similar grounds the failure of the megaspores to form cell walls might be urged against the interpretation of these cells as such, but here again this character has dropped out without, it seems to me, invalidating the view that the cells in question are spores which are thereby removed one step further from their more primitive condition in which a cell wall was eminently necessary.

To be sure we are not rid of the conclusion that, in those plants in which the embryo-sac arises directly from the mother-cell the embryo-sac is constructed, so to speak, of four spores; or that, when the mother-cell suffers one division giving rise to two daughter-cells of which one only is functional, the embryo-sac is constructed of two spores. But after all, spores, in the sense here meant, are equivalent vegetative cells of a somewhat special sort, but with no necessarily separate existence, teleologically speaking, except in those plants in which the spores are the functional equivalent of the seeds in the higher plants.

The conclusion is therefore drawn that the cells under discussion are distinguishable by their origin, that they are not cut out of the cycle of development and that their morphological persistence is connected with the function of the mitoses; while on the other hand, the spore "*as such* * * * is wanting" * because the particular features of specialization which characterize spores have been lost in connection with the loss of the more primitive spore function.

The following question is very naturally raised at this point, namely, at what point in the life cycle does the gametophyte commence its existence.† There can of course be no categorical, or at least no final answer given under our present understanding. But we may state as fairly in accord with the facts, that the morphological identity of the gametophyte has been reduced along with the change in the function of the spores, and the two periods of development have been merged into each other, to different degrees in different plants. In the most extreme cases, as *Lilium*, the gametophyte appears to commence histologically with the embryo-

* Atkinson, *l. c.* The italics are his.

† Coulter and Chamberlain in their recently published volume "Morphology of the Spermatophytes" (New York, 1901), take a tentative position. In their accounts they "close the history of the sporophyte with the appearance of the spore mother-cell," since it seemed to them "to be the best defined line of demarkation" (page iv).

sac-mother-cell. Against the adoption of such an interpretation it may be urged that, where each of four spores form the primordium potentially or actually of an embryo-sac, the gametophyte becomes spilt up into four individuals. To be sure there are analogies to come to our aid, such as the constant development of four embryos from a single proembryo or the much more widely distributed phenomena of vegetative reproduction.

But in spite of these analogies, it would seem more natural to regard the gametophyte as *an individual by coalescence*, having its origin in four like vegetative cells whose primitive function has been lost. These four cells become grafted on each other, so to speak. The chief difficulty seems to be due to our formal conceptional idea of an individual, which in nature, outside of our consciousness, does not exist.

In comparison with the cases at present before us we may recall the very important results obtained by Juel * and by Murbeck.†

Juel found that in *Antennaria dioica* a tetrad division takes place, and his account of the process differs in no essential detail from mine. In *Antennaria alpina*, however, in which parthenogenesis is now known to obtain, the tetrad division does not occur, and the embryo-sac-mother-cell develops directly into the embryo-sac without any reduction in the number of the chromosomes or the usual chromosome-ring formation. Nor is this process to be compared with that in some plants in which megaspores are not delimited in the usual manner, but in which parthenogenesis does not occur (*Lilium*). In such plants the first two divisions are the same in character as the two divisions occurring when four megaspores are formed.

In *Alchemilla*, as shown by Murbeck, parthenogenesis also occurs, and in such event the megaspore mother-cell divides somewhat irregularly, forming at most three cells, because of the failure of one of the daughter-cells to divide. These divisions are not different in character from ordinary divisions; or, in other words, the heterotypic and homotypic divisions fail, and the chromosome number remains constant.

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* Vergleichende Untersuchungen über typische und parthenogenetische Fortpflanzung bei der Gattung *Antennaria*. K. Sv. Vet. Akad. Handl. 33: No. 5. 1900.

† Parthenogenetische Embryobildung in der Gattung *Alchemilla*. Acta Reg. Soc. Physiogr. Lund. 11: No. 7. 1901.

In the light of the discovery of the fact that true tetrad mitoses do not occur in parthenogenetically reproduced plants * we are more than ever forced to insist upon the conclusions stated previously, that, in such plants as certain Liliaceae, the condition must not be interpreted as a suspension of spore formation, but as a reduction in the ontogeny of the gametophyte correlated with a loss of the spore function, without a loss of the morphological character of the spores so far as their development, as indicated by their mitoses, is concerned. The failure of true tetrad divisions in *Alchemil'a* and *Antennaria* is a notable exception which proves the rule.

The peculiarities characteristic of the tetrad divisions are now known to occur in so many plants that we may well pause to estimate their morphological significance, apart from the relation they hold to the maintenance of a constant number of chromosomes.

In general acceptation the term archesporium has signified a cell or cell aggregate from which the spore mother-cells are developed, as first defined by Goebel.†

These cells cannot, however, be distinguished from vegetative cells except by the fact that they are richer in food and plasmic content—they have the character of embryonic cells. According to their period of growth and the number of divisions they undergo their derivatives, the spore mother-cells (taken collectively the sporogenous tissue), are few or many. Now the cells of the sporogenous tissue are different from all other cells in the life cycle in that they always suffer tetrad division, and thereby exhibit very constant morphological characters which raise them to special morphological rank. This cell, or tissue, as the case may be, by virtue of the special character attaching to it, demands special recognition in terminology, and it would seem both natural and justifiable to regard it as constituting the archesporium. The use of the term in this way seems to me simple and unmistakable.

6. The third and following divisions are typical, and the reduced number of chromosomes is preserved.

In *Crucianella* all of the four megaspores in a given series may

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* Juel (*l. c.*) interprets the phenomenon in *Antennaria alpina* as a special case of apogamy.

† Bot. Zeit. 38 : 545, 561. 1880; 39 : 681, 697, 713. 1881.

and often do develop further, and undergo a simultaneous division (the first embryo-sac division), from which the actual physiological and morphological equivalence is inferred.

THE BEHAVIOR OF THE POLLEN TUBE IN DIODIA AND RICHARDSONIA

(PLATE 15)

While engaged in the foregoing study of the embryology of certain Rubiaceae, namely, *Diodia teres*, *Diodia Virginiana* and *Richardsonia pilosa*, some conditions of rather more than usual interest attaching to the behavior of the pollen tube in these forms were met with. In view of the attention which the subject has of late received in some quarters—attention which has not by any means been barren of important results—it seems well at this time to present the outcome of a closer study of the phenomena in the above-mentioned plants, although the general subject in the Rubiaceae promises as a reward of future investigation further results of interest.

The flower in both genera presents the same chief features in common. The differences are chargeable to the different numerical relations in them, *Diodia* having a bicarpellary and *Richardsonia* a tricarpellary ovary. This fact having been mentioned, we shall from this time on ignore it, since it has no bearing in particular upon the following description and for the sake of simplicity we shall take *Diodia* as the type.

MORPHOLOGICAL RELATIONS OF THE STYLE

The locules of the inferior ovary are separated by a partition of two portions, distinct in origin and in anatomical structure. The lower portion, or basal element, arises as a ridge from the floor of the ovary, and inserted on its margin are the ovules, one in each locule. This basal element reaches to somewhat less than one-half the depth of the ovary. The remaining distance is occupied by a partition—the roof element—derived from the styles which growing out laterally from the upper part of the hollowed-out receptacles join at the middle point and grow upwards and downwards to form the style. This consists therefore of two (*Diodia*) or three (*Richardsonia*) concrescent elements. The roof and floor

elements of the ovarial partition fuse at their point of meeting and so complete the separation of the originally single ovarial chamber into locules.

HISTOLOGY

When the parts above described are young, the cells are, of course, uniformly isodiametric. After all the primordia are laid down, and the period of rapid growth prior to the assumption of the definitive form sets in, the relative rapidity of growth of the several organs is accompanied by changes in the proportions of the component cells. At about the time of fertilization the histological features are as follows.

The basal element of the ovarial partition consist of thin-walled parenchyma whose elements are slightly elongated in the direction of the flower axis and contain the vascular tissue which supplies the ovules. Where the stylar portion of the partition abuts against the basal elements, the cells of the latter are flattened out transversely, this being due partly to the pressure and partly to the mode of growth. The epidermis of the basal element of the partition has, at this point, very much thickened walls. The thickening commences in the outer walls (*fig. 1*) and extends at the period of fertilization to the lateral, but not to the basal walls (*fig. 5*). At the same time the regularity of the layer of cells is lost in the process of fusion which takes place between the basal and the stylar elements of the partition.

The stylar portion of the partition is composed at the time of fertilization of three distinct tissues. The innermost of these, or that lying in the axis of the compound style, and that with which we are here concerned constitutes a conductive tissue and is composed of elongated narrow cells with transverse or slightly oblique end walls and no intercellular spaces. These cells may be further distinguished from the surrounding tissues by their denser cytoplasmic content, and thicker, more deeply staining walls. Capus (2) has noted that the cells of the conductive tissue in the plants studied by him have thickened walls (collenchyma), but in *Alchemilla arvensis* according to Murbeck (10) no such thickening is present in any part of the course of the conducting tissue, a condition which is found in the free portion of the style of the plants under consideration. Here the cells are longer than in the stylar

partition, have thin walls with somewhat thinner cytoplasm, but no intercellular spaces. The conductive tissue is readily recognized in the style proper by the smaller transverse diameters of its cells. The change from this thin-walled tissue to the thick-walled is quite abrupt and takes place at the level of the roof of the ovary.

At the lower extremity of the style, at the point namely where it fuses with the basal partition, the conductive tissue suddenly expands from being long and narrow, to short, irregular and isodiametric. There is also an evident increase in the thickness of the cell-walls. The line of fusion which may be recognized easily in the young condition as represented in *fig.* 1 is now so irregular as to be of itself unobservable. This irregularity and confusion of cells appears to be due to the thickening and partial gelatinization of the cell-walls or more especially of the middle lamella, for one may notice in sections that irregular lines of separation occur between the cells, thus indicating their loose relations. Such changes have been noted, also, by Capus (2) although according to him such changes are much more pronounced in the style and stigma So far as the writer's observation goes such pronounced changes do not occur in the upper portion of the style, although here the walls are a little thickened, and that they are on the more and more pronounced the deeper one penetrates the whole style.

At this point of fusion, as described in the previous paragraph, the ovules, two in *Diodia*, three in *Richardsonia*, are inserted upon the edge of the basal partition. The epidermis above described as undergoing a thickening of its walls is continuous with the epidermis of the ovules, a description of which is necessary at this point.

The ovule is, broadly speaking, anatropous, and possesses, in addition to the single integument characteristic of the gamopetalae, a second outgrowth, the origin of which has been elsewhere described (p. 53) as a secondary enlargement of the funicle, a sort of strophiole. A profile view of this organ, as seen in *Diodia teres*, is shown in text *fig.* 1, and as seen in *Richardsonia* in *fig.* 9. A striking feature of this organ with which we are at present concerned is the profound modification of the epidermis to form a special con-

ductive tissue for the pollen tubes. When young the cells of this tissue are of an approximately cubical or columnar form (*fig. 1*) with thin walls. As the ovule develops they change into deep columnar cells with thick walls excepting at their inner ends (*fig. 5*), where the basal and lateral walls for a little distance are thin. The contents of these cells are more dense than those of the deeper lying cells and the cytoplasm is very finely granular. Their nuclei are large with very finely divided chromatin. These cells appear to correspond in general with the cells described by Capus (2) as occurring at the base of the funicle in *Dracaena elegans* and by Dalmer (3) in the placenta of *Mahonia aquifolium*. From those in *Dracaena*, however, the cells in *Diodia teres* and *Richardsonia* differ in having thick walls, and from those in *Mahonia* in the fact that they do not secrete the mucilage which, according to Dalmer, serves as food for the pollen tube. They are not glandular cells, but, strictly speaking, cells of a conductive tissue as is shown by the behavior of the pollen tube towards it. With reference to the strophiole it may be remarked that this organ is present in other members of the same family, but that, so far as known, in no other is such a special tissue present, excepting as we shall see beyond in *Diodia Virginiana*. On the upper side of the funicle these cells have somewhat different dimensions from, but otherwise are exactly like those of the lower side where they have the longest ongitudinal diameter (*text figs. 8 and 9*). Here the strophiole lies against the ovules so as completely to close the micropyle. It will be seen that there is thus formed a collar of columnar epidermis which is continuous around both sides of the funicle and, as will be shown, completes the path of the conductive tissue from the style to the micropyle.

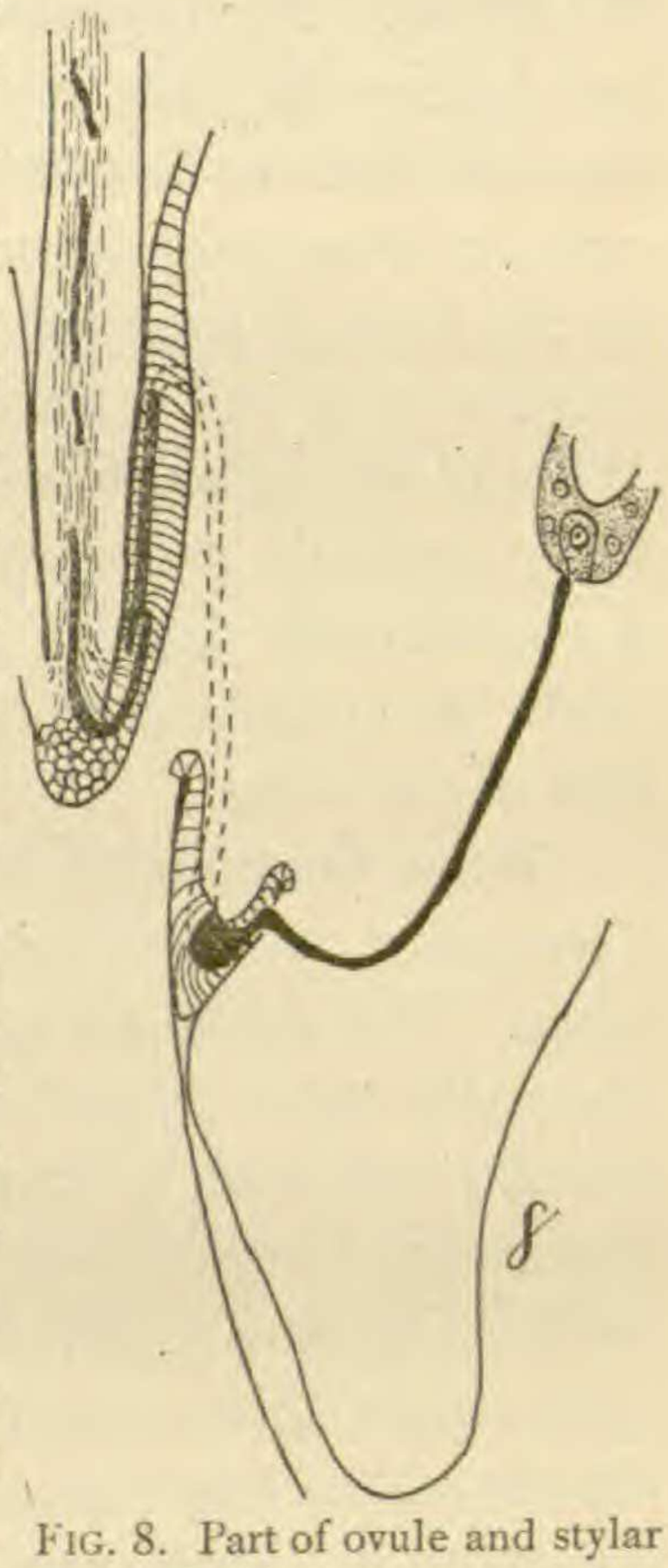

FIG. 8. Part of ovule and stylar tissue of *Diodia teres*, showing the path of the pollen tube.

THE COURSE OF THE POLLEN TUBE

In the free upper portion of the style the cells of the conductive tissue are, as above pointed out, long and very narrow. Through this tissue the pollen tube takes a somewhat devious, though on the whole direct course (*fig. 2*), and as the tube has a transverse diameter much greater than that of the neighboring conductive tissue cells, one would expect that the mechanical pressure alone would cause not a little disturbance in the conductive tissue. Such, however is not the case. The pollen tube does not destroy the cells among which it passes either by chemical influences, such as digestion, or mechanically. It is true that the cells are frequently disturbed in position, as is plentifully evidenced by the form of the nuclei when the pollen tube happens to exert pressure on a cell in such a way as to affect its nucleus (*fig. 3*). Without exception, however, the nuclei and cytoplasm stain perfectly normally and evenly. The walls of the pollen tubes are to a considerable degrees thicker than those of conducting cells, while the cytoplasm of the former is more coarsely granular than that of the latter.

When the pollen tube reaches the stylar partition it meets with conductive cells with thicker walls and of larger transverse dimensions. The nuclei are here long-oval in form. Here the path has about the same character as in the free part of the style. Occasionally one finds a sharp turn such as is shown in *fig. 3*, which represents a small part of the conductive tissue in longitudinal section showing a pollen tube cut transversely. A study of this preparation shows clearly that the effect of the pollen tube upon the tissue in this part of its course through which it passes is mechanical. Instances are frequently found in which the pollen tube passes athwart the conductive tissue, either directly or more or less obliquely. At first glance it is somewhat difficult to attribute such behavior on the part of the pollen tube to mechanical influences. When, however, we reflect upon the extreme sensitiveness of the protoplasm of the pollen tube to stimuli, it becomes less difficult. The impingement of the end of the pollen tube upon the end wall of a cell is, we believe, enough to turn it out of its direct path. Upon approaching the point of fusion of the basal and roof elements of the ovary septum the pollen tube

meets with cells of similar histological characters as those of the cells through which it has immediately passed, save in dimensions and in the mucilaginous condition of the inner lamella. Here they are irregular and isodiametric as above stated, and are derived in part from each element of the septum. Through this mass of tissue the pollen tube takes a more irregular course, showing some tendency to branch, and passes from the stylar tissue into the upper part of the basal septum, into, therefore, the epidermis above described as continuous with the columnar epidermis of the

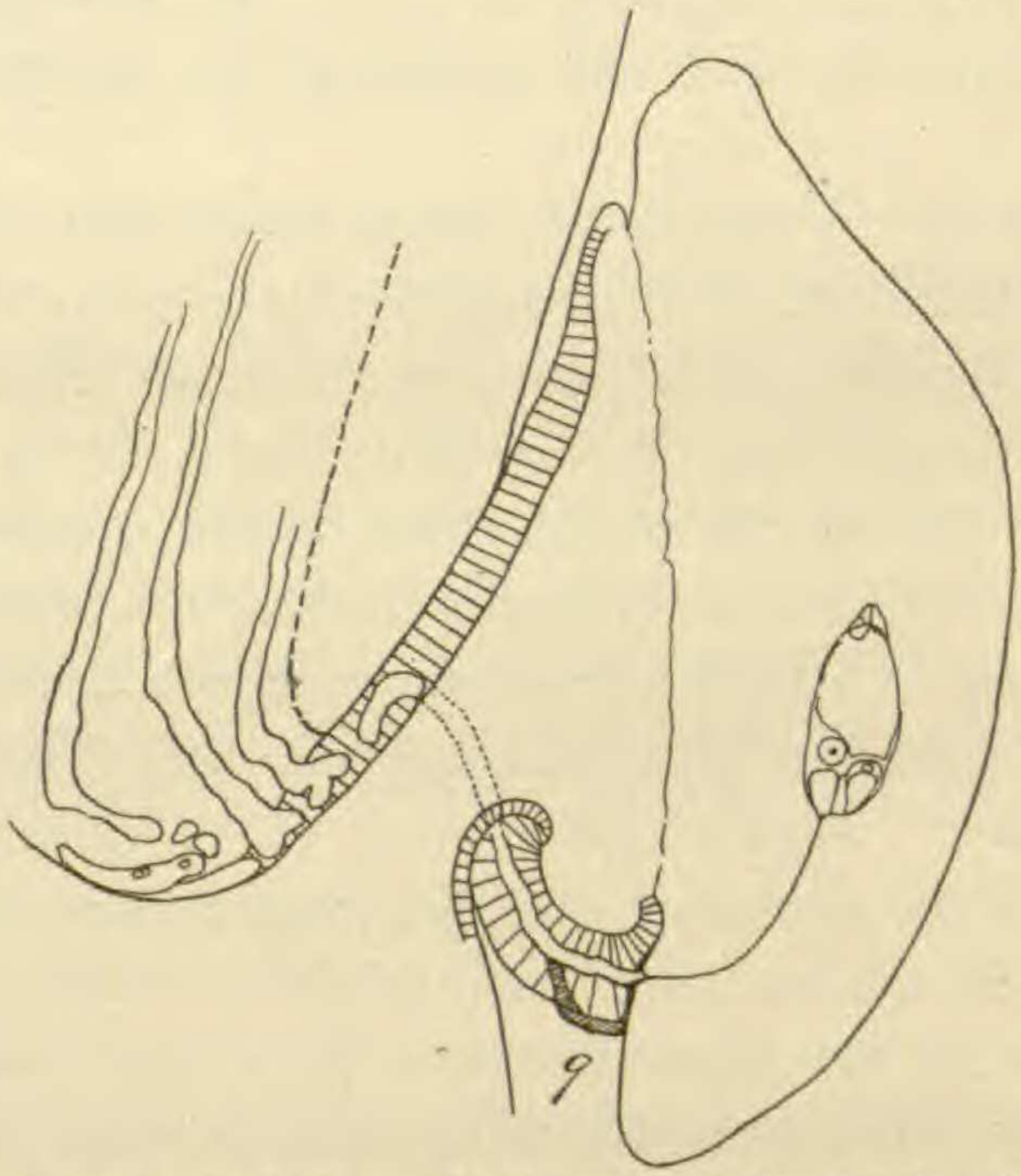

FIG. 9. Ovule and part of the stylar tissue of *Richardsonia pilosa*, showing path of the pollen tubes.

strophiole. Upon meeting with the ordinary thin-walled parenchyma of this, the tube abruptly turns at right angles to its former course (*text fig. 9*). In no case does the tube penetrate beyond the thick-walled fusion cells further than to basal septum. The contents of the pollen tube may be seen well in the lower region of the style. In properly stained preparations the generative nuclei have been repeatedly found. The vegetative nucleus, however, has not been seen in this region. The cytoplasm is densely and finely granular, entirely filling the tube, while in the older parts of the same only a thin granular layer may be discerned on the wall, or none at all.

In this part of its course the pollen tubes which are usually to be found in numbers produce a destructive effect upon the cells, upon which they impinge. The effect has the appearance of being due to mechanical pressure owing in part to the number of tubes present, and in part to their larger diameter. The contents of the conductive cells thus affected appear homogeneous and deeply staining and the nuclei lose their normal appearance, while the cells are crushed out of shape (*fig. 4*). The effect of the tube is unequal, destroying cells, now here, now there, without any dissolution of the walls, showing at once the difference between a mechanical operation, and one involving the histolysis of the circumjacent tissue as by digestion.

The behavior of the pollen tubes when they reach the subepidermal parenchyma of the basal partition is quite uniform, and is strongly suggestive of a purely mechanical influence. In the case of *Richardsonia* there is formed at that point by virtue of the confluence of the funicles of the three ovules, a basin so to speak, filled with the isodiametric irregular conductive cells. Should we plant a seedling in a basin of soil the roots would penetrate till the walls of the basin had been reached, and they would then glide along the walls. Such a case appears to be quite analogous to the one before us, excepting that geotropism plays no part in the behavior of the pollen tube. In *Diodia teres* the penetration of the epidermis of the basal partition does not take place as in *Richardsonia;* but rather the tubes bend upward in the irregular cells of the stylar conductive tissue, passing at first between the walls of the style and strophiole, but soon penetrating the columnar epidermis of the latter (*text fig. 8*).

Having now reached the thick-walled epidermis, the pollen tube travels along till it enters the regular thick-walled columnar cells which constitute the collar of connective tissue about the funicle and the strophiole. It then passes out of the fusion tissue and travels in a fairly direct path upwards, that is toward the chalazal region of the ovule (*text figs. 8 and 9*). It gradually turns, however, so as to pass around one side or the other of the funicle, and then finds itself in the epidermis of the lower side of that organ. At this time the course is more or less irregular, and the pollen tube sometimes branches (*fig. 7*). Occasionally in the

region of the micropyle it passes beyond the surface of the epithelium (*fig. 6*). It then glides along the surface a short distance and then passes between the conductive epidermis and the integument (*text fig. 1*). Ultimately it finds the micropyle and travels along it to the embryo-sac.

Two points of importance are to be noted at this point, the entirely or almost entirely intercellular path of the pollen tube, and the direction of its path which is not in the direction of least resistance, but at right angles to the longtudinal axis of the cells of the conducting tissue.

During this part of its course, that is, as it is passing through the fusion tissue and the strophiolar conductive tissue, the pollen tube as above pointed out not infrequently branches (*fig. 7*). Nawaschin has noted a similar tendency on the part of pollen tubes in the Birch (11, p. 22), to branch "wo das betreffende gewebe keine deutliche Anordnung seiner Zellen in longitudinalen Reihen aufweist." When in the strophiolar conductive tissue, the pollen tube shows more or less inability to pursue a path which is constantly at right angles to the component cells. It may then slip out to the surface. This happens most frequently in *Richardsonia* in the neighborhood of the micropyle where there is little or no pressure exerted upon the strophiole by the adjacent structures. In *Diodia teres* where the strophiole in the micropylar region is tightly wedged between the ovule and the wall of the ovary, the pollen tubes take constantly a deeper course (*text fig. 8*). These phenomena lead to the suggestion that the cause which has brought about the habit of intercellular growth in the pollen tube is a mechanical one, and this factor may probably be called upon to account for the behavior of the pollen tube in many forms. We have in mind such cases as occur, for example, in the Cannabinaceae of which Zinger (23) has given an account. According to this author, the intercellular node of growth is of phylogenetic significance, and, by means of such interpretation, brings his view into line with that of Nawaschin, who has vigorously supported the contention that the behavior of the pollen tube in the Amentiferae is inherited from their ancestral forms, and has endeavored, on this theory, to connect the lower Angiosperms with the Gymnosperms A discussion of this question is not pertinent here, but it may be

said in passing that recently Murbeck (10) has, we believe, successfully, contested the view of Nawaschin. I believe that a study of the facts in a larger number of plants will lead to a more general recognition of the principle laid down by Murbeck (10) in the words, "Der intercellulare Wachstumsmodus des Pollenschlauches ist als eine physiologische Eigenthümlichkeit zu bezeichnen, * * *" and of the *mechanical conditions* as constituting not the least important factors in leading to such behavior. As a further illustration of peculiar mechanical conditions I would cite the instance of *Cynomorium* in which, as shown by Pirotta and Longo (18), the micropyle is completely closed and there appears in its place a cone of conductive tissue through which the pollen tube must penetrate. Zinger (21) has shown somewhat similar relations in *Cannabis*.

When, as shown above, the pollen tube passes to the surface, it travels a short distance along the conductive tissue, soon finding its way between the strophiole and the integument. Under such circumstances the wall of the pollen tube may well be seen (*fig. 6*).

The effect of the pollen tubes upon the cells of the collar of conductive tissue appears to be purely mechanical, as in the case of that in the style. Forcing its way between the cells, the pollen tube causes in them but little or no distortion and, so far as I have observed, no destruction (*figs. 5, 6*). It would appear that, although the cells of the conductive tissue form a true tissue, and are not crowded papillae, their thickened walls are nevertheless not bound together firmly but rather are loosely related by a soft, mucilaginous middle lamella.

Having reached the micropyle, the pollen tube now passes into the micropylar canal. The cells lining the latter are characterized by somewhat thicker, more deeply staining walls than those of the other integumental cells. The pollen tube eventually reaches the synergids. In the plants under discussion these cells have the "hyaline, striated tips," as described by Strasburger (21), extremely well developed. The generally accepted view that the synergids serve as a conductive apparatus is strengthened by the analogy which I venture to believe exists between their curious cellulose tips and the thickened and partially gelatinized cell walls of the conductive tissue.

Diodia Virginiana

In this species the conductive tissue is of a somewhat different character from that in the two plants already discussed. In the style both above and below the roof of the ovary the cells are cylindrical, their transverse walls being transverse, though occasionally oblique in varying degrees. The nuclei are not so much elongated, and correspondingly the length of the cells is not so great. The cell walls are thin throughout the whole range of stylar conductive tissue, excepting near the region of fusion of the basal and stylar parts of the partition where a slight thickening of the walls occurs. The conformation of the stylar conductive tissues is the same as in the other species.

In the character of the conductive tissue of the ovule, however, lies the greatest difference, a difference which, as we shall see, is connected with the behavior of the pollen tube. The tissue is disposed on the strophiole in a band around the insertion of the funicle, and extending downwards towards the micropylar region. The accompanying text figure 10 shows in a schematic way the arrangement. The component epithelial cells are approximately cubical in form (*fig. 8*), are fairly richly supplied with cytoplasm, and have a large nucleus. The outer walls are thickened, and exactly correspond in all respects to the homologous tissue in the other species studied in the young condition, before any further changes take place, *i. e.*, before the cell elongates into columnar form. At the time of pollination, a mucilaginous secretion appears so that at this time there lies on the surface of the conductive tissue a layer of mucilage of a thickness of nearly half the depth of the cells. The mucilage may be traced between the cells to some depth.

This conductive tissue corresponds in general to that which has been described by Dalmer (3) as occurring on the placenta in certain Liliaceae both in form and in the occurrence of the mucilaginous secretion through which that author has shown that the pollen tubes grow, and from which they derive nutrition which enables them to grow onward toward the micropyle. Apparently similar cells have been described by Lang (5) in *Byblis* and *Polypompholyx*.

The course of the pollen tube through the style needs no remark since what has been said above applies throughout. One point, however, needs mention, namely, that here the cellulose plugs described by Treub (**22**) as occurring in the pollen tubes in *Casuarina*, by Nawaschin (**12, 16**) in the Amentiferae and by Murbeck (**10**) in *Alchemilla* are here very easily and clearly seen. Two of these plugs are shown in *figs. 10 and 11*. It will be seen from the outline of the plug and the form of the somewhat shrunken pollen tube contents that these fit each other. One finds sometimes that the tube on one side of the plug is empty (*fig. 10*). In other cases cytoplasm occurs in both sides (*fig. 11*). Similar plugs occur lower down in the fusion tissue, but they have not been observed elsewhere.

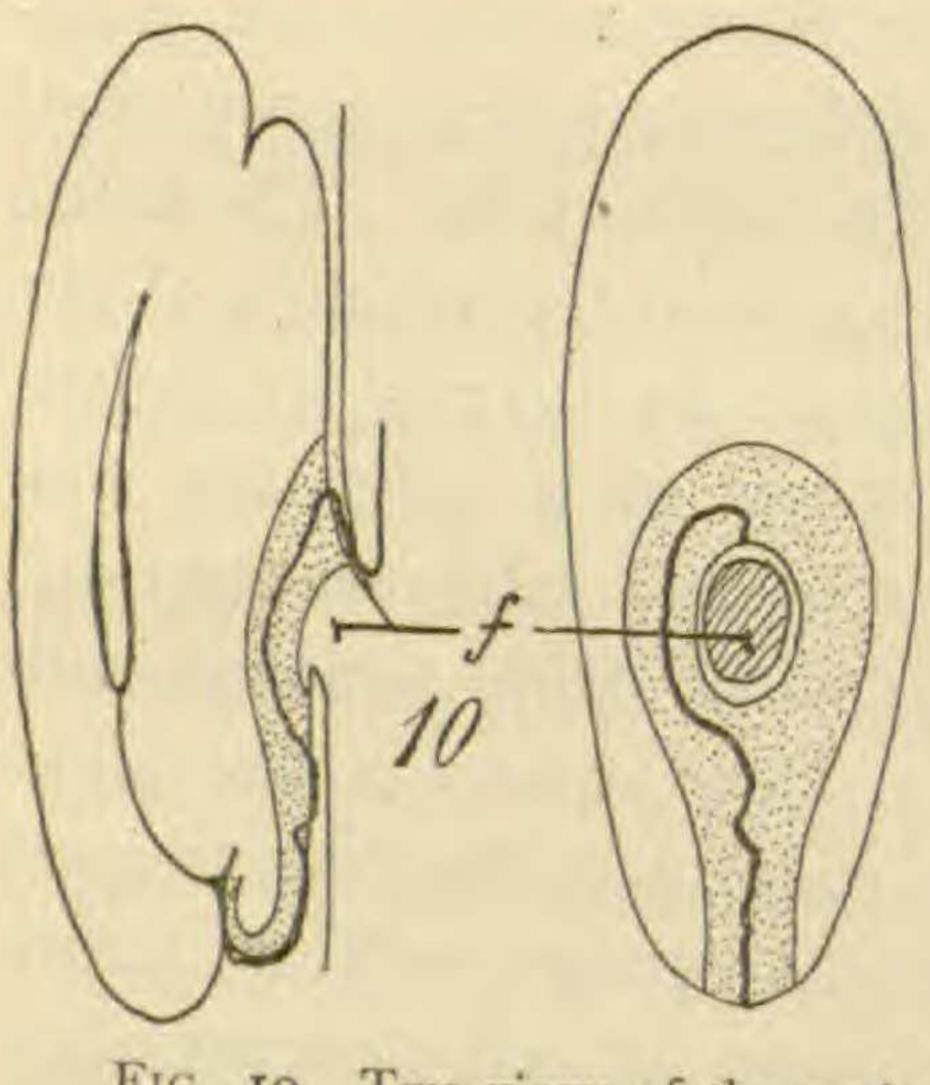

FIG. 10. Two views of the ovule of *Diodia Virginiana*, showing the conductive epidermis (dotted) and the path of the pollen tube; *f*, funicle. (*Schematic.*)

When the pollen tube reaches the epidermis of the basal partition (*text fig. 10*), it stops at that point, and does not enter between the cells as occurs in *Richardsonia*. They then bend upward and travel between the two tissue masses until they come to the collar of conductive epidermis of the strophiole, in the mucilaginous outer wall of which they pursue their way. After moving upward, *i. e.*, toward the chalaza, a little distance, they turn, following the band of secreting epidermis as shown in *text fig. 10*, and being completely buried in the mucilage, as occurs in plants studied by Dalmer (**3**). The behavior of the pollen tube toward the walls of the secreting cells needs particular mention in view of the fact that the wall of the former coalesces with the outer walls of the latter in such a way as to be completely indistinguishable. In one preparation the pollen tube was cut transversely at one point of its course and showed this relation especially well. In *fig. 9* this is well shown, from which it will be seen that the outer part of the tube wall is thin, thickening markedly on the sides nearer the epi-

dermis, and, as above said, coalescing with its outer wall. It is not clear, however, whether the pollen tube wall as represented, and as it appears to the eye in the preparation belongs, properly speaking, entirely to the pollen tube. At any rate the soft character of the walls and their coalescence makes the determination of this point difficult.

When the pollen tube reaches the lower end of the strophiole, it travels, as before, superficially around and into the rift between this and the integument. This rift is filled with the slimy secretion derived entirely from the epidermis of the strophiole, inasmuch as the epidermis of the integument is not secretory. In this way the micropyle is at last reached and entered.

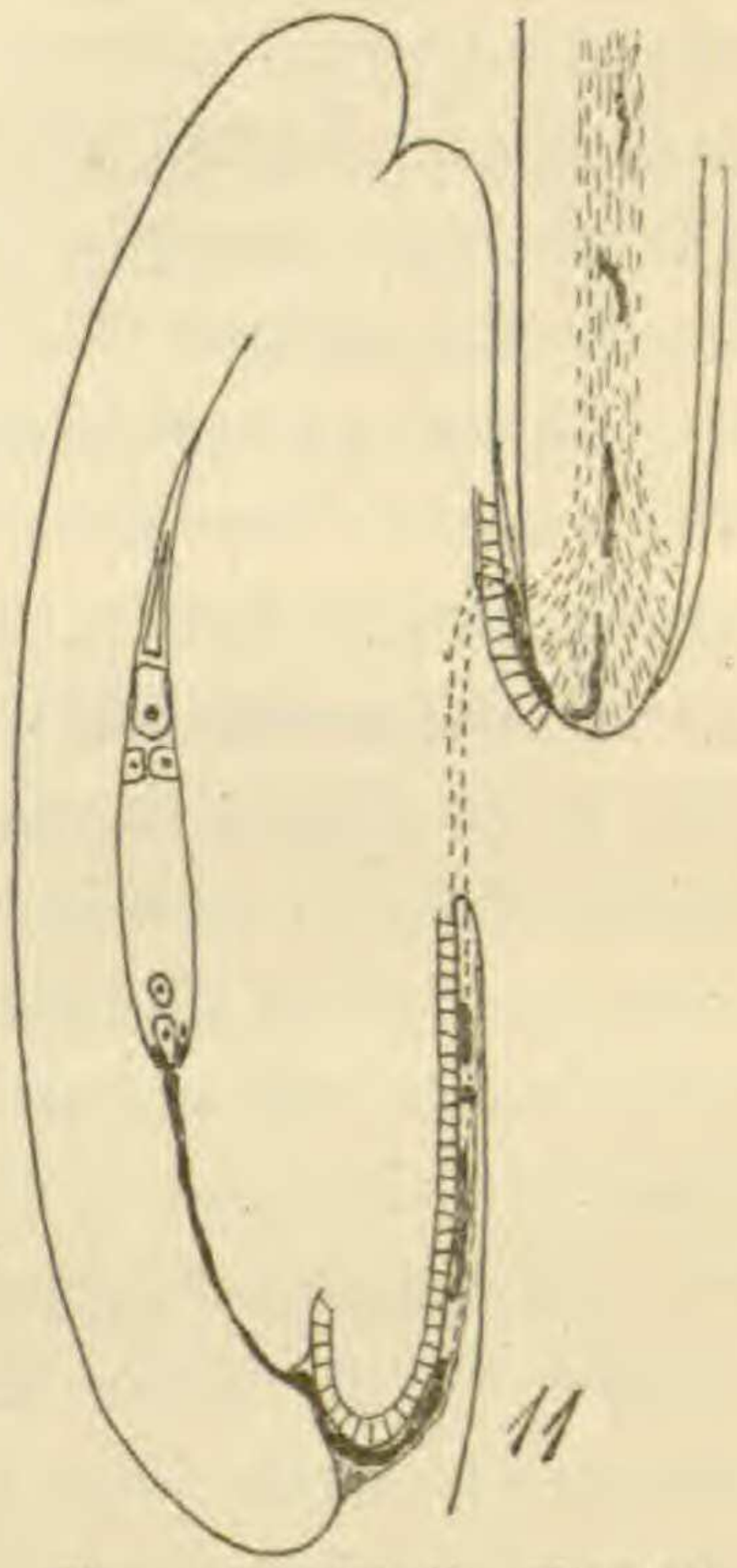

FIG. 11. *Diodia Virginiana.* Ovule and portion of the style, showing the path of the pollen tube.

If now we compare the three types described as regards the development of the strophiolar conductive tissue, and the behavior of the pollen tube, we may accordingly arrange them into a series in the following order and with the characters indicated.

1. *Diodia Virginiana.*—The conductive tissue consists of epidermal cubical cells with thickened outer walls covered with a mucilage secreted by them. The mechanical relations of the ovary are such that the pollen tubes may easily lie between the strophiole and the ovary wall. The pollen tube travels *superficially* along the funiculus and strophiolar conductive tissue, but imbedded in the mucilaginous secretion.

2. *Diodia teres.*—The conductive tissues consists of columnar epithelial cells with thickened outer and lateral walls. The middle lamella is mucilaginous. There is no secretion on the outer surface. The mechanical relations are such that the strophiolar conductive tissue is compressed between the ovule and ovary wall. The pollen tube enters the said conductive tissue immediately on commencing its upward movement along the funicle and remains within till it reaches the micropyle.

3. *Richardsonia pilosa.*—The conductive tissue as in *Diodia teres*, but somewhat different histological relations exist in the fusion tissue of the ovary septum. The mechanical relations are such that the strophiolar conductive tissue above the funicle is compressed between the ovary wall and the ovule. Below, *i. e.*, toward the micropyle, the pressure is reduced. The pollen tube enters the conductive tissue at the base of the stylar portion of the partition, travels by the intercellular method in the same till it reaches the micropylar region where it often though not always bends towards the surface.

The above-named facts speak for the mechanical conditions in the ovary as important factors in determining the course of the pollen tube, whether intercellular or not.

In review the following results and conclusions are presented:

1. In *Richardsonia pilosa* and *Diodia teres* the course of the pollen-tube is chiefly intercellular, a phenomenon clearly secondary in view of the fact that in a closely related species, *Diodia Virginiana*, the pollen tube pursues its path freely in the ovary cavity in that part of its course.

An important factor in determining such behavior of the pollen tube is found in the mechanical relations (pressure) existing between the ovule and the ovary wall. This is added as evidence supporting the contention of Murbeck to the effect that the intercellular mode of growth of the pollen tube is a matter of physiological meaning.

Inasmuch as such a difference in the behavior of the pollen-tube as has been pointed out may occur between two closely related species of the same genus, I infer, contrary to the view of Nawaschin, that the behavior of the pollen tube is of very limited significance—indeed of no practical significance—from a phylogenetic viewpoint. The condition is analogous to that of the archesporium in the angiosperms, which occurs, so far as at present known, in eight genera of the Rosaceae and in a dozen others widely scattered through the whole range of forms from the Rubiaceae (5, 6) to *Casuarina*.*

This statement does not preclude the possibility that Nawas-

* A summary of the facts and literature pertaining thereto will be found in papers by Murbeck and Koernicke already quoted.

chin's contention, that the primitive behavior of the pollen-tube was entirely intercellular, is true.

2. The conductive tissue of the style consists of: (1) very thin-walled cylindrical cells in the upper part and (2) thicker walled cells in the lower part. The latter pass from a cylindrical to an irregular isodiametric form in the fusion tissue, in which the middle lamella is evidently soft and yielding.

3. A conductive tissue is here described which is morphologically a part of the ovule, or, more exactly, a part of a peculiar secondary outgrowth which I have designated the strophiole. It takes two histological forms: (1) In *Diodia Virginiana* it is composed of cubical, mucilage secreting cells, with thickened outer walls, corresponding to analogous cells found in many plants on the placenta. (2) In *Diodia teres* and *Richardsonia pilosa* the homologous tissue is composed of columnar, thick-walled cells (the thickening being absent from their bases only) with a soft middle lamella. No outer mucilage layer is here found.

In these plants we find a conductive tissue composed of elongate, regular cells, in which their longitudinal axes are placed at right angles to the intercellular path of the pollen tube for the direct passage of which this condition is mechanically less favorable than in the kind of conductive tissue in which the longitudinal axes of the component cells are parallel to the path of the pollen tube.

4. The pollen tube does not in general act unfavorably upon the cells with which it comes into contact. When however it does it may be referred to mechanical causes. This conclusion appears to accord with the observations of others.

5. The pollen tube shows a tendency to branch where the cells are irregular and isodiametric, or where the long axis of the conductive tissue cells are placed at right angles to the course of the pollen tube. This behavior speaks against the view of Miyoshi: "Im Griffel werden die Pollenschläuche wesentlich mechanisch zum Fruchtknoten gelenkt" (8).

6. There remains an undetermined factor in the guidance of the pollen tube. We cannot tell how the same is able to find its way to the egg in the definite way in which it does. The pollen tube is negatively aërotropic, and is chemotropic, as shown by

Molisch (9). These facts explain its behavior toward the stigma, but the later behavior in the conductive tissue remains unexplained. According to Molisch again, chemotropism " muss bei der Wanderung des pollenschlauches zur Eizelle, resp. bei der Auffindung derselben in vielen Fällen eine wichtige Rolle zufallen." But it is difficult to see how chemotropism can be an efficient factor unless there be a differential distribution of the stimulant. Miyoshi on the other hand, has maintained, in the words above quoted, that the operation is a purely mechanical one. Nawaschin (16), to whom we are indebted for a critical exposition of these two views, has pointed out that both presuppose that " der Pollenschlauch Reizbewegungen ungehindert ausführen könne," a condition which is found only where the pollen tubes have free space in which to move. If we assume that such are the conditions in *Diodia Virginiana*, in which the pollen tube moves freely in the ovarial cavity, what may we say of *Diodia teres* and *Richardsonia pilosa*, in which the pollen tube is not free to move in the ovarial cavity, but pursues an intercellular path, parallel to that which we may believe, from the case of *Diodia Virginiana*, it would take were the mechanical conditions favorable, and this in spite of the character of the conductive tissue which is mechanically unfavorable.

I believe that we may be justified in maintaining:

1. With Nawaschin, that the occurrence of a secreting conductive tissue speaks as much for the nutrition of the pollen tube as for the necessity of mechanical guidance; that, moreover, the ability of the pollen tube to secrete a cellulose-dissolving enzyme indicates the former, as in point of fact the pollen tube does not penetrate the cells of the conductive tissue; and that the assumption that in order to respond to a stimulus, the pollen tube must have unoccupied space in which to move is unnecessary and probably untrue.

2. With Molisch, that chemotropism is the important factor, and the guidance of the pollen tube; but that *the distribution of the stimulant in the conductive tissue is a differential one;* and, as Nawaschin (16) suggests, " die Verbreitung der Reizstoffe Kann zwischen den Papillen des Leitgewebes in Folge der capillarkräfte leicht zu Stande kommen," extending his statement, however, to cells as well as papillae.

The experiments of Miyoshi (8) by which he showed that pollen tubes will penetrate the cut end of a style and travel toward the stigma, and that when a lateral cut is made into the conductive tissue the pollen tubes will grow out in various directions, we maintain not only do not support his view that the pollen tubes are guided in a purely mechanical way, but also that they do not contradict the suggestion of such a differential distribution of an appropriate stimulant, inasmuch as a cut in the conductive tissue would in all probability set up disturbances by which the ordinary distribution of the stimulant might be reversed.

It may, of course, be that Miyoshi's contention is true for the style, although I believe that the case is not proven ; but that the pollen tube is guided in one way in one region of the conductive tissue, and in another way in another, seems quite improbable.

3. The suggestion is submitted that the synergidae are the source of the stimulant, and constitute the center of distribution of the stimulant. To this idea I am led by the cytological character of those cells, and by their position as the only effective center, exclusive of the egg itself. We do not forget the experiment of Strasburger (**21**) on *Torenia*, the results of which were, however, negative. Searching experimentation is still needed to throw light on the perplexing problem which we have attempted somewhat further to elucidate.

△ A List of Literature pertaining to the Pollen Tube.

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